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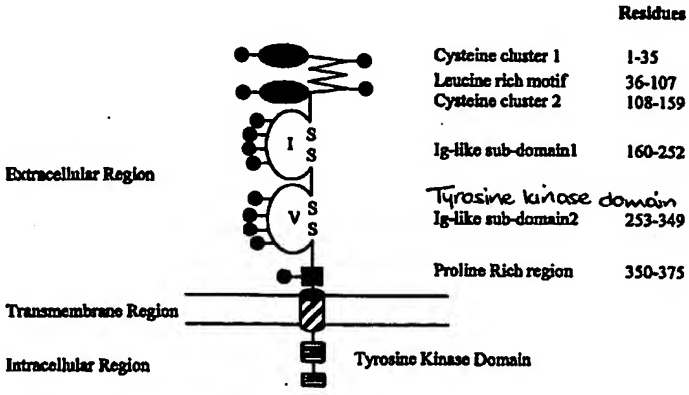

(57) Abstract

This invention relates to the use of a domain of Trk as a therapeutic agent and for screening purposes and rational design of NGF mimetics.

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THERAPEUTIC AGENT FOR NGF

This invention relates to therapeutic agents and screening methods. In particular, the invention relates to the use of the Ig2 domain of the tyrosine kinase TrkA and fragments thereof in the treatment of disorders in which levels of neurotrophins, such as NGF, are elevated such as in pain disorders. It also relates to the use of the TrkAIg2 domain as a target for screening for compounds which act to antagonise or to mimic the actions of neurotrophins such as NGF. TrkAIg2 is defined here as including the TrkAIg-like sub-domain 2 together with the proline rich region (Fig. 1A).

Nerve Growth Factor (NGF) is a potent neurotrophic factor for forebrain cholinergic neurones and promotes the survival and differentiation of sympathetic and sensory neurones during development. In animal models it has been shown that administration of NGF is able to correct the effects of cholinergic atrophy in aged or lesioned animals. Purified mouse NGF has been used as a treatment for Alzheimer's disease. This treatment, however, requires invasive surgery and a long term solution would be the generation of small molecule agonists able to mimic the trophic actions of NGF. NGF usually exists as a dimer, however, for these purposes, the term NGF embraces monomeric dimeric, trimeric, or heterodimeric forms.

Evidence suggests that NGF may also act as a mediator of some persistent pain states (McMahon S.B. Series B-Biological Sciences, (1996), Vol.351, No.1338, 431- 440) by interacting with receptors on nociceptive primary afferents. In a variety of experimental inflammatory conditions NGF levels are rapidly increased in the inflamed tissue. Similarly, the systematic or local application of exogenous NGF produces a rapid and prolonged behavioural hyperalgesia in both animals and humans. In a number of animal models, much of the hyperalgesia associated with experimental inflammation is blocked by molecules which are able to sequester NGF, including antibodies. Therefore

peripherally acting NGF - sequestering agents or NGF antagonists may potentially be used in treating some chronic pain states.

Peripheral inflammation is usually characterised by heightened pain sensitivity or hyperalgesia, which is the consequence of the release of inflammatory mediators, cytokines and growth factors. NGF seems to play a central role in pain mediation through its action on the TrkA receptors of a sub-group of the nociceptive sensory neurons of the dorsal root ganglion (DRG). In the adult this comprises some 40% of DRG cells. These neurons also express the peptides Substance P and calcitonin-gene related peptide (CGRP). By the action of NGF on TrkA receptors there results an increase in neuropeptide levels in these sensory neurons; in addition sodium and calcium channels are affected such that these neurons are increased in excitability. These actions may result in an increase in pain levels. Thus, NGF sequestering agents such as the TrkA extracellular domains may potentially be used to reduce these pain levels.

Under conditions of continual NGF up-regulation, chronic inflammation may lead to a persistent pain state. There are various models of chronic inflammation which involve exogenous administration of NGF or its upregulation. One such model (Woolf, C.J. *et* al. British Journal Of Pharmacology, (1997), Vol.121, No.3, 417- 424) is that induced by intraplantar injection of complete Freund's adjuvant in adult rats. This produces a localized inflammation of the hindpaw with elevation in the levels of TNF β, IL-1 β and NGF. TNF α injections have been reported to produce an increase in thermal and mechanical sensitivity which is attenuated by prior administration of anti-NGF antiserum. Carrageenan administration is known to cause a specific increase in NGF mRNA levels (of up to 500%) which is not seen for other neurotrophins such as NT-3 and BDNF.

In chronic inflammatory states the effects of consistently elevated levels of NGF may result in a long-term disabling pain state. Examples of this may be in some forms of bladder cystitis where raised levels of NGF have been found in biopsies (Lowe, E. M. *et al* British Journal Of Urology, (1997), Vol.79, No.4, 572-577). A rat model of human chronic cystitis, induced by administration of an irritant chemical can be treated, again by NGF sequestration, by administration of TrkA immunoadhesin (Dmitrieva, N. *et al* Neuroscience, (1997), Vol.78, No.2, 449-459). Systemic treatment with the

NGF-sequestering molecule was able to partially and significantly reverse established inflammatory changes, by about 30-60%. The administration of exogenous NGF into the lumen of the urinary bladders of normal rats also has been shown to produce a rapid and marked bladder hyper-reflexia similar to that seen with experimental inflammation. It is also likely that chronically increased NGF levels may lead to both peripheral sensitization of nociceptors and central sensitization of dorsal horn neurons and perhaps even long-term sensory neuronal abnormalities (McMahon, S. B. Series B-Biological Sciences, (1996), Vol.351, No.1338, 431- 440).

In arthritic synovial fluid, high levels of NGF have been observed. Transgenic arthritic mice have also been shown to have raised levels of NGF and an increase in the number of mast cells (Aloe, L. *et al* International Journal Of Tissue Reactions-Experimental And Clinical Aspects, (1993), Vol.15, No.4, 139-143). Purified NGF antibodies injected into arthritic transgenic mice cause a reduction in the number of mast cells, as well as a decrease in histamine and substance P levels within the synovium (Aloe, L. *et al.* Rheumatology International, (1995), Vol.14, No.6, 249-252).

It seems likely also that the postherpetic neuralgia (PHN), associated with the disorder shingles, may involve upregulation of NGF protein. Varicella-zoster virus (VZV) is an α herpes virus responsible for two human diseases: chicken pox in childhood (varicella), and shingles. The virus remains latent in dorsal root ganglia and may re-emerge later in life, taking advantage of the decline in immune function that occurs with aging. Reactivation causes herpes zoster, commonly known as shingles. The incidence of herpes zoster increases with advancing age. Pain, allodynia, and sensory loss in the affected dermatome are the central manifestations of the disorder. Severe pain is the major cause of acute and chronic morbidity in patients with herpes zoster. The chronic and often debilitating pain, PHN, is the most common complication of herpes zoster. Up to 50% of elderly patients who have had shingles may develop PHN. Antiviral agents appropriately administered systemically greatly relieve the pain of acute shingles, also antidepressants maybe useful; conventional analgesics however are generally of little use, though in a few patients some relief has been obtained with opioids, particularly methadone. The difficulty with testing the effects of anti-NGF treatment is that the model for shingles is not possible in the rat, there is only a cat model. However, it may be possible to investigate such treatments in

human subjects, with the potential for reduction of NGF levels and alleviation of associated pain.

Chronic inflammatory conditions are widespread and current therapies are severely limited. For instance it is estimated that arthritis affects 37.9 million people and interstitial cystitis 450,00 people in the United States. In a study of rheumatoid arthritis, more than 80% of the patients were in severe pain despite the fact that the majority were taking analgesics. Similarly, there is no effective therapy for interstitial cystitis, which is characterised by painful bladder symptoms.

NGF is one of a family of neurotrophins involved in the development and maintenance of the peripheral and central nervous system. NGF may be isolated from various sources, most particularly from male mice salivary glands. It may be isolated first as 7S NGF, named for its sedimentation coefficient, which is a complex of β-NGF and γNGF. 2.5S NGF may be obtained from this. 2.5S NGF is known to be responsible for the neurotrophic biological activity of the complex. 2.5S NGF is βNGF but often partially proteolysed at the amino and carboxy termini. The other members include for example BDNF, NT-3 and NT-4. All of the neurotrophins bind to a common receptor p75NGFR. Each also binds to one of a homologous family of tyrosine kinase receptors: NGF binds to TrkA, BDNF and NT-4 bind to TrkB, and NT-3 binds to TrkC. NT-3 can also bind TrkA and TrkB with reduced affinity.

Although the three dimensional structure of the TrkA extracellular domain is unknown, distinct structural motifs in the sequence have been characterised (Figure 1A). The Trk extracellular domain comprises three tandem leucine rich motifs (LRM), flanked by two cysteine cluster regions, followed by two immunoglobulin-like (Ig-like) domains. Based on sequence homology with the neural cell adhesion molecule and the platelet derived growth factor (PDGF) receptor, the Ig-like domains have previously been classified as belonging to the C2 class of the immunoglobulin superfamily (IgSF) (Williams, AF, and Barclay AN (1988) Ann Rev Immunol 6, 381-405). Numerous studies have defined neurotrophin residues which interact with p75NGFR and Trk receptors but little is known about the Trk residues which are involved in binding the neurotrophins.

Recently two groups have shown that the Ig-like domains of the Trk receptors play important roles in the binding of neurotrophin ligands and receptor activation. Perez P. *et al* (Molecular and Cellular Neuroscience 6: 97-105 (1995)) concluded that both of the Ig-like domains are important for the binding of NGF to TrkA. Urfer, R. *et al* (EMBO J. 14 p2795-2805 (1995)) concluded that the second Ig-like sub-domain and proline rich region, Ig2 (Figure 1A) provide the main contacts for NGF binding.

The extracellular domain of TrkA is 375 amino acids long. The inventors have recently shown that a protein comprising the two immunoglobulin-like domains and proline-rich region (amino acids 160-375) alone are able to bind NGF with a similar affinity to that of the complete extracellular domain (Holden P. H *et al* (1997) Nature Biotchnology 15: 668-672). This region has been defined here as TrkAIg1,2. Surprisingly, the inventors have found that an even smaller domain of TrkA referred to as TrkAIg2 (amino acids 253-375) is able to bind NGF with a similar affinity to the complete extracellular domain or the TrkAIg1,2 region and is thus responsible primarily for its binding properties.

The inventors have demonstrated that the recombinant Ig-like domains are able to bind neurotrophins such as NGF with high affinity and inhibit the biological activity of NGF *in vitro* and *in vivo*. In particular, TrkAIg2 as defined by amino acids 253-375, (Figure 1A) is the major contributor to NGF binding. The inventors have used molecular modelling techniques to model the TrkAIg1 and TrkAIg2 domains. Surprisingly, they find that TrkIg2 - like sub-domain 2 is not of the C2 class but of the V set of Ig-like domains (Figure 1B).

This gives rise to several uses for TrkAIg2 and polypeptides derived therefrom. Structural data from co-crystals of TrkAIg2-NGF will identify the residues in TrkA which are involved in binding NGF. This will enable rational design of neutrophin, particularly NGF, mimetics. Immobilised TrkAIg2 can be used as a target for phage display libraries as well as combinatorial chemical libraries and fungal extracts. This will allow for selection of molecules able to bind TrkA and thus either act as agonists or antagonists at the receptor. A third use of TrkAIg2 is as a therapeutic agent for a number of chronic pain states. NGF is particularly important for peripheral sensory neurones, evidence suggests that NGF may act as a mediator of some persistent pain states by interacting with receptors

on nociceptive primary afferents and that peripherally acting NGF antagonists may be of use in treating some chronic pain states such as rheumatoid arthritis, interstitial cystitis and shingles.

A first aspect of the invention provides a polypeptide comprising the amino acid sequence of residues 22 to 119 of Fig. 4(B) or a portion of the amino acid sequence of Figure 4(B), and which binds a neurotrophin. Preferably, the polypeptide consists of the whole sequence of amino acids 22-144 of Figure 4(B). The polypeptide may be TrkAIg1,2 or a portion thereof. Such a polypeptide may be produced by chemical or biological means.

We exclude the full coding sequence of natural TrkA.

The polypeptide may be derived from animal cells. More preferably, the polypeptide is selected from mammalian cells, and in particular, may be selected from human cells. Alternatively, the polypeptide may be selected from avian cells including chicken cells or reptile or amphibian or fish or insect.

Preferably, the neurotrophin is NGF, NT-3, or a neurotrophin which binds p75 NGFR. Such a neurotrophin may exist in a monomeric, dimeric, trimeric or heterodimeric form, and may be from a mammalian, such as a human.

A second aspect of the invention provides a DNA sequence encoding a polypeptide according to a first aspect of the invention; or variants of such a DNA sequence due to the degeneracy of the genetic code, or insertion or deletion mutants thereof that encode a polypeptide according to a first aspect of the invention, and DNA sequences which hybridise to such a DNA sequence. This DNA sequence may be inserted into a plasmid or other vector such as pET15b.

A further aspect of the invention provides a complex comprising a polypeptide according to a first aspect of the invention in combination with at least one neurotrophin or neurotrophin subunit, such as NGF or NT-3.

A further aspect of the invention provides a method of producing a polypeptide according to a first aspect of the invention comprising introducing a DNA sequence according to a second aspect of the invention into a suitable host and cultivating that host whereby the TrkAIg2 is expressed. A suitable host may be selected from animal cells such as bacterial cells, insect cells and mammalian cells, particularly human cells.

Further, the TrkAIg2 may be conveniently used as a target for a high throughput screen for molecules which bind to the TrkA receptor using a polypeptide according to a first aspect of the invention. Such a method may involve the use of phage or peptide display libraries, combinatorial chemical libraries and fungal extracts, and ELISA techniques.

A futher aspect of the invention comprises comparative binding of a putative ligand to at least a portion of TrkAIg1 with its binding to at least a portion of TrkAIg2. Such methods may involve selecting molecules which bind to at least one solvent exposed loop of TrkAIg2, such as the E to F loop or C" to D loop as shown in Fig. 1(B). The molecules selected may enhance the binding of a polypeptide according to a first aspect of the invention, or at least a portion of TrkA in its natural state, to a neurotrophin.

A further aspect of the invention provides a method of combinatorial chemistry comprising generating compounds and screening the compounds using their binding affinities to a polypeptide according to a first aspect of the invention.

A further aspect of the invention comprises an antibody raised against a polypeptide according to a first aspect of the invention, particularly TrkAIg2.

A further aspect of the invention comprises a host cell containing a polypeptide according to a first aspect of the invention carried on a plasmid. Such as host cell may be mammalian (including human), bacterial, insect, yeast, avian, amphibian, fish or reptilian.

A further aspect of the invention comprises a diagnostic probe comprising a portion of a polypeptide according to a first aspect of the invention. The probe may be labelled with a fluorescent tag or radiolabel.

A further aspect of the invention comprises diagnostic tests, assays, or monitoring methods using a polypeptide according to a first aspect of the invention, particularly in the detection of elevated neurotrophin levels.

A further aspect of the invention comprises an organism engineered to express a polypeptide according to a first aspect of the invention.

A further aspect of the invention comprises a method of treating a subject with pain associated with increased neurotrophin polypeptide levels, the method comprising supplying to the subject a pharmaceutical composition comprising a polypeptide according to a first aspect of the invention, or an NGF analogue isolated or identified by a screeening procedure as described above.

The pain may be a symptom of ISU, interstitial cystitis, arthritis, shingles, peripheral inflammation, chronic inflammation, or postherpetic neuralgia.

A further aspect of the invention comprises a treating a subject of Alzheimer's disease comprising supplying to the subject a pharmaceutical composition comprising a polypeptide according to a first aspect of the invention, or a composition comprising a neurotrophin analogue isolated or identified by a screening procedure involving a polypeptide according to a first aspect of the invention.

A composition comprising a polypeptide according to a first aspect of the invention can be used to reduce free NGF levels in a subject.

All references above to neurotrophin embrace NGF and NT-3.

A further aspect of the invention includes a homology model having the coordinates shown in Fig. 21, and machine readable data storage medium on which such a homology model has been stored, and a computers programmed with, or arranged to provide such a homology model.

A further aspect of the invention provides crystalling TrkAIg2.

A further aspect of the invention provides compounds obtained by a method as mentioned above, using a computer as mentioned above, or using a machine readable data storage medium as mentioned above.

A further aspect of the invention comprises a crystal comprising a polypeptide according to a first aspect of the invention, particularly a TrkAIg2 polypeptide.

The invention will now be described, by way of example only, with reference to the accompanying drawings Figures 1 to 20 in which

Fig. 1 (A) is a schematic representation of the TrkA structure (the filled circles represent consensus glycosylation sites);

Fig. 1(B) shows a modelled structure for TrkAIg1 and TrkAIg2; the most important binding determinates probably occur in the loop connecting strands E and F (the EF loop).

Fig. 2(A) is a restriction map of the plasmid pET15b;

Fig. 2(B) shows the sequence of oligonucleotides used to amplify TrkAIg1,2.

Fig. 3 shows the nucleotide sequence of the insert of pET15b-TrkAIg1,2 and its derived amino acid sequence;

Fig. 4(A) shows the nucleotide sequence and derived amino acid sequence of his TrkAIg1;

Fig. 4(B) shows the nucleotide sequence and derived amino acid sequence of his TrkAIg2;

Fig. 4(C) shows the TrkAIg2 domain of a splice variant of TrkA including the six amino acid insert in the proline-rich region able to bind NT-3;

Fig. 5 is a gel illustrating expression of TrkAIg1,2, TrkAIg1 and TrkAIg2;

Fig. 6(A) is a gel illustrating purification of TrkAIg2;

Fig. 6(B) is a gel illustrating purification of TrkAIg1;

Fig. 7(A) shows an elution profile of TrkAIg1 from Poros 20HQ after refolding;

Fig. 7(B) shows an elution profile of TrkAIg2 from Poros 20HQ after refolding

Fig. 8 shows a Circular Dichroism spectrum of TrkAIg2. The molecular ellipticity (θ) is shown as a function of wavelength.

Fig. 9 shows competitive binding Assay for TrkAIg1,2 and TrkAIg2; The axis is given in logarithmic scale as 1×10^{-11} to 1×10^{-5} M.

Fig. 10 shows surface plasmon resonance (SPR) of NGF binding to Immobilised TrkAIg2;

Fig. 11 illustrates the results of binding experiments where TrkAIg2 (2μM) and TrkAIg1 (2μM) were incubated separately with a standard curve of βNGF (0-1000pM);

Fig. 12 illustrates the results of binding experiments where increasing concentrations of βNGF (1-200μM) were incubated separately with 2μM TrkAIg1 or 2μM TrkAIg2;

Fig. 13 shows the effect of TrkAIg2 on NGF dependent neurite outgrowth on PC12 cells.

Fig. 14 A to F illustrates the effect of co-injected TrkAIg1,2 on NGF-induced plasma extravasation;

Fig. 15 illustrates the effect of 5 minute pre-treatment with TrkAIg1,2 on NGF- induced plasma extravasation;

Fig. 16 illustrates the effect of 40 minute pre-treatment with TrkAIg1,2 on NGF-induced plasma extravasation;

Fig. 17 illustrates the effect of co-injected TrkAIg1 on NGF-induced plasma extravasation;

Fig. 18 illustrates the effect of co-injection of TrkAIg2 on NGF-induced plasma extravasation;

Fig. 19 illustrates the effect of 5 minute pre-treatment with TrkAIg2 on NGF-induced plasma extravasation;

Fig. 20 illustrates the effect of 40 minute pre-treatment with TrkAIg2 on NGF-induced plasma extravasation.

Fig. 21 shows the coordinate data for the model of Fig. 1 (B).

Structure prediction of the extracellular domain of TrkA and modelling of the Ig-like domains:

Secondary structure analysis of the Ig-like regions using PredictProtein (Rost B. and Sander C. (1993) PNAS **90**: 7553-7562; Rost B. and Sander C. (1993) J. Mol. Biol. **232**: 584-599; Rost B. and Sander C. (1994) Proteins **19**: 55-72) showed defined stretches of β-strands. The first Ig-like sub-domain, TrkAIg1, consists of residues 160-252 (Fig. 1A) in the mature extracellular domain of TrkA, while the second Ig-like sub-domain consists of residues 253-349 (Fig. 1A). There is also a proline rich region at residues 349-375 (Fig. 1A).

For TrkAIg1, two known proteins (parents) were identified as homologues from which a model could be built. These are 2NCM (domain 1 of mouse NCAM) and 1VCA (domain 1 of human vascular cell adhesion molecule). Both domains are I-set Ig domains and have 32% and 29% sequence identity, respectively, with the target sequence. 2NCM was identified as the most suitable parent on which to base the model, apart from residues 38-50 connecting β-strand C to D where the smaller loop found in 1VCA was used (Figure 1B).

For TrkAIg2, two parents were identified as homologues from which a model could be built. These are 1TNM (titin module M5) and 1HNG (CD2 domain 1). The homologues are quite distantly related at 21% and 14% sequence identity and belong to the Ig-set I family and the V set family respectively. However, certain key features of the Ig fold can be identified including a disulphide bridge and a Trp in the C strand. This is surprising since both homologues lack a disulphide bond. These homologues show higher sequence identity in different regions, hence a chimeric model was built using 1TNM as the main

template and 1HNG being used to model residues 39-59 (Figure 1B) and the coordinate data is shown in Fig. 21.

Following slight manual interventions in the sequence alignment the inventors have elucidated a model containing 8 β-strands with strands (ABDE) in one sheet and (A'CFG) in the other sheet. Together they form the β-sandwich for TrkAIg1. For TrkAIg2, the A' strand is absent and two extra strands C' and C'' are predicted with the β-sandwich formed by β-strands (ABDE) and (GFC'C"). For domain 1, the alignment mapped the disulphide between strands B and F across the β-sandwich to the same position as found in 2NCM. This disulphide also superimposed onto the 1VCA disulphide between residues 23-71. Conversely for domain 2, a disulphide is predicted on the surface of the molecule bridging two adjacent β-strands, B and E, the second Cys aligns with a Ser in 1TNM. This disulphide bond arrangement is similar to the model predicted by Urfer *et al* (Urfer, R., Tsoulfas, P., O'Connell, L., Hongo, J.A., Zhao, W. and Presta, L.G. (1998). J. Biol.Chem. Urfer *et al. (supra)* 273: 5829-5840) modelled on 1VCA domain 1 although our TrkAIg2 model predicts nine β-sheets, of the V-set, in contrast with the model with seven β-sheets in a I-set arrangement. The modelled structures are shown in Figure 1B and the co-ordinate data is shown in DATA.1.

In terms of the structural model built here for TrkAIg2 the parents used in model construction, titin module M5 (1tnm) and CD2 domain 1 (1hng) are clearly distant homologues, that can be identified by sensitive sequence search methods (Barton, G.J. (1993) Comput. Appl. Biosci. 9: 729-734; Henikoff, S. and Henikoff, J.G. 1991. Nucleic Acids Research 19: 6565-6572). The VCAM domain 1 used to model build TrkAIg2 by Urfer *et al.* (Urfer, R., Tsoulfas, P., O'Connell, L., Hongo, J.A., Zhao, W. and Presta, L.G. (1998) JBC 273: 5829-5840 is not significantly related by sequence, however, is homologous by virtue of being an Ig-fold. Relative to titin and VCAM (both I-set domains) the TrkAIg2 sequence has a significant insertion (~10 residues) between strands C and D. The region corresponding to positions 39-59 which includes this insert has more significant homology to CD2 domain 1 than other Ig domains. Furthermore, the predicted secondary structure (Rost B. and Sander C. (1993) PNAS 90: 7553-7562) of TrkAIg2 in this region corresponds to the existence of two extra strands (C' and C'') in accordance

with the CD2 structure. This results in a predicted V-set domain as opposed to the I-set domain proposed by Urfer *et al.* (Urfer, R., Tsoulfas, P., O'Connell, L., Hongo, J.A., Zhao, W. and Presta, L.G. (1998). JBC 273: 5829-5840)

The importance of key residues in binding NGF can be understood by reference to our model and the extensive mutational analysis of TrkAIg2 by Urfer *et al.* (Urfer, R., Tsoulfas, P., O'Connell, L., Hongo, J.A., Zhao, W. and Presta, L.G. 1998. J. Biol.Chem. **273**: 5829-5840). The most important binding determinants in TrkAIg2 occur in the loop connecting strands E and F (the EF-loop) with single mutations T319A, H320A and N323A exhibiting greater than 100-fold reduction in binding. Reference to our structural model indicates that all three residues are in solvent exposed locations near the apex of the EF-loop. Minor contributors to loss in binding affinity also occur in the spatially adjacent AB-loop with mutations H258A, V261E, M263A and H264A. The first three residue locations are in solvent exposed locations on the surface of this loop. Only two other mutations exhibit greater than 50-fold reduction in binding affinity, these are P269E and H310A. These two residues are spatially adjacent to one another in our model and in close proximity to the disulphide bridge (C267-C312) connecting strands B and E. It is possible these residues play a direct role in binding NGF as suggested by Urfer *et al.* (Urfer, R., Tsoulfas, P., O'Connell, L., Hongo, J.A., Zhao, W. and Presta, L.G. 1998. J. Biol.Chem. **273**: 5829-5840). However an alternative explanation may be their importance in maintaining the structural integrity of the disulphide bridge. Unlike the conserved core disulphide bond of canonical Ig domains the solvent exposed disulphide bridge may not be important in stabilising the structure of the domain, however, the covalent link between strands B and E may be important in maintaining the conformation of the AB and EF loops in binding. Indeed the loss of the disulphide with mutations C267A or C312A results in a 10 to 30-fold reduction in binding, underlining the importance of the disulphide bridge in the binding mechanism.

An alternatively spliced form of TrkA containing a six amino acid insert (at amino acid position 224-225 (Fig. 3)) in the proline rich domain, VSFSPV, shows a higher affinity for NT3 and therefore may be important for ligand binding (Clary, D. O & Reichardt L. F. (1994) PAISA **91**: 11133-11137). This sequence is also found in the rat TrkA sequence and a similar sequence is found in the chicken TrkA. There is also a similar of polar residues in all of the TrkB sequences (Allen S. J. *et al.* (1994) Neuroscience **60**: 825-834).

It is therefore possible that this region may contribute to the binding of the neurotrophins or to the receptor's specificity.

The TrkAIg1,2 region is generally considered as comprising amino acids 160-375 of the mature extracellular domain of TrkA (Fig. 1A), TrkAIg1 or TrkAIg like sub-domain 1, as comprising amino acids 160-252 and including TrkAIg - like subdomain 2 as amino acids 253-349. TrkAIg2 here comprises amino acids 253-375 the proline rich region. In all cases the use of variants of TrkA and its sub domains such as those described above are embraced by the present invention.

Construction of TrkAIg2 with the Insert from the Alternatively Spliced Variant:

TrkAIg2 with the insert from the alternatively spliced variant was created by PCR mutagenesis. The mutagenesis was done in two stages. First the 5' and 3' fragments were amplified such that there is an overlap encoding the sequence of the alternative spliced form of TrkA. In the second stage, the PCR products of the 5' and 3' fragments were spliced together using the overlapping sequence and the two flanking primers. The first round of PCR involved oligo66816 (ATCATATGCC GGCCAGTGTG CAGCT) and oligo49234 (CCACTGGCGA GAAGGAGACA GGGATGGGGT CCTCGGGG) to produce the 5'-fragment and oligo49233 (GTCTCCTTCT CGCCAGTGGA CACTAACAGC ACATCTGG) and the T7 terminator primer (GCTAGTTATTGCTCAGCGG) to produce the 3'-fragment. The products were then purified and used as target for a second round of PCR using oligo66816 and T7terminator primer. The PCR product from the second round of PCR was then cloned into pET15b and expressed in the same way as TrkAIg2.

Sub-cloning of TrkAIg1,2:

From the secondary structure prediction data, it was decided to subclone the DNA encoding amino acids 160 to 375 (Fig.1A)of the extracellular domain of TrkA. Oligonucleotide primers (10692 and 10693) were designed that would provide appropriate restriction sites in order that the TrkAIg1,2 insert would be in-frame with the poly-histidine tag of the expression vector, pET15b (Novagen) and two stop codons to terminate translation. A map of pET15b and the sequence of the oligonucleotide primers is shown in Figure 2.

Amplification by PCR was then carried out using the primers oligo10692 and oligo10693 (Cruachem Ltd) and the full-length Human TrkA cDNA clone (a gift from David Kaplan, Montreal Neurological Institute, Canada) as target. The PCR product was then ligated into the plasmid pCRII (Invitrogen), to give pCRII-TrkAIg1,2. pCRII-TrkAIg1,2 was then digested with *Xho*I and the insert purified from a low-melting point agarose gel by phenol extraction and ligated into pET15b (Novagen) previously prepared by digesting with *Xho*I and dephosphorylating using Calf-Intestinal Alkaline Phosphatase (CIAP). After transformation into *Escherichia coli* XL1Blue (Stratagene), transformants were screened by PCR using the T7 promoter primer which anneals to pET15b and oligo10693. In this way, clones were identified which had the TrkAIg1,2 insert in the correct orientation for expression from the T7 promoter. The resulting clone, pET15b-TrkAIg1,2 was sequenced from the T7 promoter primer and the T7 terminator primer to ensure that the insert had ligated to the pET15b at the *Xho*I site. The DNA sequence of the insert of pET15b-TrkAIg1,2 and the derived amino acid sequence are shown in bold in Fig. 3 (amino acids 24-239, nucleotides 71-718). Enzymes and enzyme buffers were obtained from Boerhinger.

Sub-cloning of TrkAIg1:

An oligonucleotide primer was designed which would allow amplification of the TrkAIg1 domain using the left primer for TrkAIg1,2 such that the PCR product could be ligated into the XhoI site of pET15b in-frame with the poly-histidine tag.

oligo36770 *Right Primer For TrkA Ig1;*

```
cgctcgag tta  tca GAAGGAGACGTTGACC
  XhoI  STOP STOP
```

Amplification by PCR was then carried out using oligo10692 and oligo36770 with pET15b-TrkAIg1,2 as target. The PCR product was then ligated into pCRII (Invitrogen) to give pCRII-TrkAIg1 which was then digested with *Xho*I and subjected to low melting point agarose gel electrophoresis. The insert was then purified and ligated into pET15b previously digested with *Xho*I and treated with CIAP. After transformation into *E. coli* XL1Blue, transformants were screened by PCR using oligo10692 and the T7 terminator primer. The resulting clone pET15b-TrkAIg1, was then sequenced to ensure that the

reading frame of TrkAIg1 was in-frame with the poly-histidine tag of pET15b. Figure 4a shows the nucleotide sequence (residues 71-349) and deduced amino acid sequence (residues 24-116) of TrkAIg1, in bold.

Sub-cloning of TrkAIg2:
An oligonucleotide primer was designed which would allow amplification of the TrkAIg2 domain using the T7 terminator primer of pET15b-TrkAIg1,2;

oligo66816 *Left Primer For TrkA Ig2;*
atcatatgCC GGCCAGTGTG CAGCT
*Nde*I

Amplification by PCR was then carried out using oligo66816 and the T7 terminator primer with pET15b-TrkAIg1,2 as the template DNA. The PCR product was then digested with *Nde*I and *Bam*HI and ligated into pET15b previously prepared by digestion with the same enzymes and treated with CIAP. Transformants were screened by PCR using the T7 promoter primer and oligo10693 and the positive clones were sequenced. Figure 4b shows, in bold, the nucleotide sequence (residues 65-433) and derived amino acid sequence (residues 22-144) of TrkAIg2.

Hybridisation to TrkA DNA sequence
DNA encoding TrkAIg1,2 or TrkAIg2 (sequences according to Figures 3, and 4B) may be used for a hybridization assay. A DNA sequence encoding TrkAIg1,2 or TrkAIg2 or portions of such a sequence may be obtained by reverse transcriptase PCR of genomic DNA or directly by PCR or restriction digest from the cDNA for TrkA. DNA or RNA which is complimentary to the DNA encoding TrkAIg1,2 or TrkAIg2 or portions of such a sequence, or a sequence which is similar in composition but contains a degeneracy of sequence, may be hybridized to the DNA prepared above. Such a sequence is referred to herein as a probe. Usually, the complimentary DNA or RNA is tagged by radioactive or non-radioactive substances.

One example of this is the northern analysis of TrkAIg2 using a radioactively labelled cDNA probe. A cDNA probe is random primed (Stratagene, CA) with ^{32}P-dATP (6000Ci/mmol; Dupont NEN). The probe is then purified using a Nuctrap column (Stratagene), to a specific activity in the region of 2 x 10^6 cpm/ng. Chinese hamster ovary

cells (CHO) expressing TrkA are then homogenised in Ultraspec™ (Biotecx, Houston Texas) and total RNA extracted. The RNA is loaded onto a 1% denaturing agarose gel and seperated by electrophoresis, before being blotted onto Hybond N (Amersham, Cardiff, UK) overnight and baked for 2 hours at 80°C. These Hybond N filters are pre-hybridized for 4 hours at 65°C by revolving in hybridization buffer (6SSC, 5 x Denhardts, 0.5% SDS and 0.002% acid cleaved salmon sperm DNA), in a hybridization oven. The probe is then denatured for 5 minutes at 100°C, before being added to fresh hybridisation solution. Filters are then hybridized under these conditions of high stringency, overnight at 65°C. Stringency may be varied according to degeneracy of probe or homology of target. Lower temperatures such as 50°C, and higher salt concentrations, such as 20x55C, will allow for lower stringency. The presence of formamide decreases the affinity of nucleic acid binding and allows for variance in stringency. Such strategies are well described (e.g. Nucleic acid hybridisation, a practical approach edited by Hames and Higgins, IRL Press 1988). The next day, the filters are washed in 2 x SSC/ 0.5% SDS and washed twice for 30 minutes at 65°C in Hybaid with 2 x SSC/ 0.5% SDS. The filters are then dried and exposed to Hyperfilm (Hyperfilm MP, Amersham) overnight, at -70°C, and developed the following day. DNA probes which have bound to RNA encoding the TrkAIg2 sequence are visualised as exposed, black, areas of the autoradiographic film.

A further example of this is the detection of expression of TrkAIg1,2 or TrkAIg2, or a similar sequences in an expression library. A λGT10 human brain cDNA library (M Goedert, Cambridge) is used to infect *E. coli* c600 cells. These are plated onto 24cm x 24cm agar plates to give 10,000pfu per plate. A plaque lift is then carried out by laying Nylon membrane Hybond N (Amersham, Cardiff, UK) onto the agar plate for 1 minute. The filter is then placed, DNA side up, on denaturing solution (1.5N NaCl, 0.5N NaOH) for 30 sec, before being immersed for 2 minutes. The filter is then immersed into neutralising solution (1.5N NaCl, 0.5N Tris-HCl pH 8.0) for 5 min. Immersion is repeated in fresh neutralising solution. The filter is then rinsed briefly in 2 X SSC (0.3N NaCl, 0.03N $Na_3Citrate$, pH 7.0) and placed on filter paper which is baked at 80°C for 2 hours. Hybridization is carried out as described above. The position of DNA probes which have bound to plaques encoding the TrkA sequence is visualised as exposed, black, areas of the autoradiographic film. These exposed, black areas can be re-aligned to the plates to identify

positive clones expressing sequences similar to TrkAIg1,2 or TrkAIg2 or a portion of such a DNA sequence.

Hybridisation may also occur using homologous PCR techniques. Specific or degenerate oligonucleotides corresponding to a region in the sequence for TrkAIg1,2 may be used to amplify a portion of the sequence as described for example, in the section entitled 'sub-cloning of TrkAIg2'. Such hybridization assays may be used as tools to detect the presence of TrkAIg1,2 or TrkAIg2 sequences, or portions thereof, in diagnostic kits.

Expression of TrkAIg1,2, TrkAIg1 and TrkAIg2:

Competent BL21(DE3) cells were transformed with the above vector and expression was carried out using a variation on the method described in the pET (Novagen) manual for difficult target proteins. Briefly, 2 ml of 2YT broth (containing 200mg/ml carbenecillin) was inoculated with a colony and grown at 37°C to mid log phase. Cells were not centrifuged and resuspended in 2YT (as in manual) but used directly to inoculate 50 ml of 2 YT broth (containing 500 mg/ml carbenecillin) and grown at 37°C to mid log phase. The cells were not harvested by centrifugation and resuspended but used directly to infect 5 litres of 2 YT (containing 500 µg/ml ampicillin). Once an OD_{600} of 1 was reached the cell culture was induced by the addition of IPTG to a final concentration of 1 mM and the cells were grown for a further 2 hrs at 37°C. Figure 5 shows a 15% SDS PAGE gel of extracts of cultures of BL21(DE3) containing the various pET15b-TrkAIg constructs. Further analysis of the cell extracts revealed that for all of the constructs, the expressed TrkAIg protein was insoluble. Several attempts were made to express the TrkAIg protein in the soluble fraction, but were unsuccessful. However, the fact that the TrkAIg proteins were insoluble faciliated in their purification.

Purification and Refolding of TrkAIg1,2:

The harvested cells were resuspended in 10% glycerol, frozen at -70°C and the pellet was passed 3 times through an Xpress (BioX, 12 ton psi). The lysed cells were washed with 20 mM Tris-HCl (pH 8.0) and centrifuged for 30 min at 10,000 rpm at 4,°C until all soluble matter was removed, leaving inclusion bodies containing insoluble protein. The purified

inclusion bodies were solubilised in 6M urea buffer (20 mM Tris-HCl pH 8.5, 1 mM β-mercaptoethanol) at approximately 0.1 mg/ml protein and incubated on ice with gentle shaking for 1 hour. Refolding was carried out by dialysis against 400x buffer (20 mM Tris-HCl, 100 mM NaCl, pH 8.5) for 24 hrs at 4°C, with one buffer change. The refolded TrkA-Ig1,2 protein was loaded onto a 1ml Resource Q (Pharmacia) column and eluted with a linear gradient of 0-1M NaCl in 20 mM Tris-HCl over 40mls at 2 mls per minute. The main peak as detected at 280 nm (using a UV detector) was collected and affinity purified according to the Novagen His column purification protocol using a 2.5 ml disposable column of His-bind resin (Novagen). Finally, the eluted protein was re-applied to the Resource Q column to remove imidazole. This was eluted with a 10 ml salt gradient of 0-1m NaCl in 20 mM Tris buffer pH 8.0.

Purification of TrkAIg1 and TrkAIg2:

The harvested cells were resuspended in 10% glycerol, frozen at -70°C and the pellet was passed 3 times through an Xpress (BioX). The extract was then centrifuged at 10,000 rpm, 4°C for 30min to pellet the insoluble inclusion bodies. The inclusion bodies were then washed in 50 ml 1%(v/v) Triton X-100, 10 mMTrisHCl pH8.0, 1 mM EDTA followed by 50 ml 1M NaCl 10mMTrisHCl pH8.0, 1 mM EDTA and finally 10 mM TrisHCl pH8.0, 1 mM EDTA. The inclusion bodies were then solubilised in 20 mM Na Phosphate, 30 mM Imidazole, 8 M Urea pH7.4. The solubilised inclusion bodies were then clarified by centrifugation before loading on a 5 ml HisTrap column (Pharmacia). The column was washed with 50 ml 20 mM NaPhosphate, 30 mM Imidazole, 8 M Urea pH7.4 and the purified TrkAIg1 and TrkAIg2 eluted with 25 ml 20 mM NaPhosphate, 300 mM Imidazole, 8 M Urea pH7.4 at 2 mls/minute (Figure 6(A) and 6(B)).

Refolding of TrkAIg1 and TrkAIg2:

The purified TrkAIg proteins were adjusted to a concentration of 0.1 mg/ml in 20 mM NaPhosphate, 30 mM Imidazole, 8 M Urea pH7.4 with the addition of 1 mM β-mercaptoethanol and dialysed against 20 mM TrisHCl, 50 mM NaCl, pH8.5 for TrkAIg2 and 20 mM TrisHCl, 50 mM NaCl pH9.0 for TrkAIg1 (2x100 volumes). The dialysed proteins were loaded onto a 1.6 ml Poros 20HQ column and eluted with a linear gradient of 0.05-1 M NaCl over 20 column volumes (Figure 7).

Three peaks were eluting from the Poros 20HQ column for TrkAIg2, all of which gave a band corresponding to TrkAIg2 (data not shown). Therefore the refolding process must result in three species of TrkAIg2, all of which have a different conformation. Displacement binding studies reveal that the first peak to elute binds NGF while the others do not. The first peak was therefore collected, glycerol added to a final concentration of 20% (v/v), and snap frozen in liquid nitrogen before storage at -70°C.

For TrkAIg1, only two peaks elute from the Poros 20HQ column with more protein in the flow through. Again SDS page of each peak and the flow through show that TrkAIg1 is the only protein present. Displacement binding assays of the two peaks show that neither of these species of TrkAIg1 bind to NGF (data not shown).

Circular Dichroism Studies on TrkAIg2

To determine the secondary structure content of the folded protein, far-UV circular dichroism (CD) measurements were made. The CD of proteins is primarily the CD of the amide chromophore, which begins absorbing far into the UV region with the first band at about 220 nm. Antiparallel β-sheet structures typically display a negative Cotton effect with a minimum near 218 nm and a positive effect with a maximum around 195 nm. The amplitude of the far-UV spectra of different immunoglobulins such as light chain variable (VL) and constant (CL) domains also show a minimum around 215-218 nm. Similar results were therefore expected with the TrkAIg proteins.

CD spectra were recorded at room temperature on a Jobin Yvon CD6 instrument using a cuvette of 0.5mm path length at a protein concentration of 40μM. Ten scans were accumulated with a scan speed of 0.5nm/s. Spectra were averaged and the small signal arising from the buffer was subtracted. The CD of the active TrkAIg2shows a minimum at 218nm and a maximum near 200nm (Figure 8). This is typical of anti-parallel β-sheet, which display a negative Cotton effect with a minimum near 218nm and a positive Cotton effect with a maximum at around 195nm (Yang, J.T., Wu, C.S.C. and Martinez, H.M. (1986). Methods Enzymol. **130**: 208-269). Similar results have been reported for other immunoglobulin domains (Ikeda, K., Hamaguchi, K. and Migita, S. (1968) J. Biochem. **63**: 654-660) and for TrkAIg1,2 (Holden, P.H., Asopa, V., Robertson, A.G.S., Clarke, A.R., Tyler, S., Bennett, G.S., Brain, S.D., Wilcock, G.K., Allen, S.J., Smith, S. and

Dawbarn, D. (1997) Nat. Biotechnol **15**: 668-672). These results are consistent with the model of TrkAIg2 shown in Figure 1B.

Thus the CD data indicates that TrkAIg2 eluting first from the Poros 20HQ column is folded into a compact structure and is likely to have a similar structure to the other immunoglobulin domains.

The binding of NGF to Immunoglobulin-like Domains of TrkA

1 Competitive Binding

The binding affinity of ^{125}I-NGF to the Ig-like domains of TrkA was determined by a competitive binding assay using the melanoma cell line A875 American Tissue Culture Collection (ATCC) which expresses the NGF receptor $p75^{NGFR}$.

Purified recombinant human NGF was radioiodinated with I^{125} using a lactoperoxidase method and equilibrium binding with [^{125}I]-NGF was carried out (Treanor *et al.*, 1991; *Neuroscience Letters* **121** p73-76). Briefly A875 cells (10^6 per ml) were incubated with [^{125}I]-NGF (0.14 nM) and serial dilutions of unlabeled human NGF (concentration range: 10^{-6} M to 1 x 10^{-11}M), TrkAIg1,2 (concentration range: 4 x 10^{-6} M to 1 x 10^{-11} M) or TrkAIg2 (concentration range 5 x 10^{-6} M to 1 x 10^{-11}m). Tubes were shaken vigorously at room temperature for 1 hr. 100 µl aliquots were then layered over 200 µl sucrose (0.15 M in binding buffer) in Beckman tubes. After centrifugation (15 seconds at 20,000 g) bound and free [^{125}I]-NGF were separated by freezing the tubes in liquid nitrogen and determining the bound [^{125}I]-NGF of the cell pellet. Binding reactions were carried out in triplicate. Counts were corrected for background and specific binding was between 85-87% of total binding. The competitive binding assay (figure 9) allowed estimation of the binding affinity of [^{125}I]-NGF to the recombinant TrkAIg2 protein. A range of concentrations of Ig-like domains are incubated with ^{125}I-NGF and A875 cells (Vale R. D. & Shooter E. M (1985) Methods in Enzymology **109**: 21-39). This results in a competition

between the TrkAIg domains and the $p75^{NGFR}$ for available ^{125}I-NGF. Two competing equilibria are:

Kdl Kd2

$$N + R \rightleftharpoons N{:}R \text{ and } N + T \rightleftharpoons N{:}T$$

where N represents NGF; R the $p75^{NGFR}$ cell receptor and T the TrkAIg2 domain.

The data represent the NGF bound to the cell at varying TrkAIg2 concentrations, as a fraction of that bound in the absence of TrkAIg2. Owing to the high affinity of NGF for the $p75^{NGFR}$ cellular receptor, the analytical solution to the curve is complex thus data were fitted using numerical simulation (FACSIMILE, U.K.A.E.A).

The fitted value for the dissociation constant for the TrkAIg1,2/NGF interaction (K_d2) was 3.3 nM (Holden *et al.*, 1997; Nature Biotechnology **15** p668-672). This agrees well with a K_d of between 0.1 and 1.0 nM. for NGF binding to ectopically expressed TrkA in mammalian cells. The IC_{50} (concentration of cold NGF required to inhibit ^{125}I-NGF by 50%) for unlabelled (cold) NGF was 0.2nM (Holden, P. H *et al.* (1997) Nature Biotechnology **15**: 668-672) (Figure 4B).

Results show that TrkAIg2 binds NGF with a similar affinity to TrkAIg1,2 (Fig. 9). The IC_{50} for TrkAIg2 is only three-fold higher than that of TrkAIg1,2, indicating a very similar affinity for NGF. This surprising result indicates that the major contribution to binding within TrkAIg1,2 is found in the second Ig domain, TrkAIg2.

2 Surface Plasmon Resonance Studies:

Kinetic data of the binding of NGF to TrkAIg2 was obtained using a BiaCore-X. Biacore technology allows real-time measurements of rate constants using very low amounts of protein. Briefly, varying concentrations of sample (analyte) are flowed across a sensor chip to which the protein of interest (the ligand) has been bound. As the analyte binds to the

ligand there is a change in the electron density on the surface of the sensor chip which affects the intensity and wavelength of light absorbed by the surface.

Since the data from competitive binding assays indicated that TrkAIg2 was the major contributor to NGF binding, this domain was further investigated.

TrkAIg2 was covalently attached to the surface of the sensor chip by coupling with amine groups on TrkAIg2 to carboxyl groups on the surface using BiaCore Amine Coupling kit and varying concentrations of NGF passed over at a constant flow rate of 20 µl/min for two minutes. Data were collected for a range of NGF concentrations of 1 µM to 1 nM. It was found that at the high concentrations and at the very low concentrations, the data became difficult to interpret possibly due to aggregation of the NGF at the high concentrations and to non-specific interactions with the surface at very low concentrations. However, data collected for the range 40 nM to 500 nM could be successfully evaluated. Using the fitting software, BiaEval 3.0, a Kd of 11.8 nM was obtained. The K_d value of 11.8 nM obtained is consistent with the fact that the IC_{50} for TrkAIg2 is three fold higher than that of TrkAIg1,2 given that the K_d for TrkAIg1,2 binding to NGF is 3.3 nM as determined by competitive binding assay.

In addition, 20 µM BDNF was also passed over the TrkAIg2 with negligible observed binding. It is clear that as well as being the main contributor to the NGF binding capability of TrkA, TrkAIg2 is also specific for NGF.

3 Binding of TrkAIg-like domains using the ELISA Technique

Method 1

Anti-βNGF (Sigma polyclonal rabbit anti mouse NGF, 1:1000) diluted in Coat I Buffer (50 mM sodium carbonate pH 9.6, NaN3 0.1%) is plated (50 µl per well) onto 96 well plates and left overnight at 4°C. Wells were emptied and 100 µl per well Coat II Buffer (Coat I plus 1% BSA) was added. After 2 hours at 4°C, the plate was washed 3 times using Wash Buffer (50 mM Tris HCl pH 7.2, 200 mM NaCl, 0.1% Triton X-100, 0.1% NaN_3, 0.25% gelatin) and samples and standard curve of NGF (0-1000pg/ml) diluted in Sample Buffer (Wash buffer plus 1% BSA) were added (50 µl per well). Samples had been

pre-incubated with varying concentrations of TrkAIg-like domains for ten minutes with shaking at room temperature before adding to the plate. The plate was left one hour at room temperature before washing 3 times with Wash Buffer, anti βNGF galactosidase conjugate (Boerhinger: 2.5-20mU and 5-10ng antibody per assay) diluted (1:40) in wash buffer (50 µl per well was added). The plate was incubated for 2 hours at room temperature and then washed 3 times with Wash Buffer before adding 50 µl of substrate (200 mM of 4-methyl umbelliferyl galactoside (4-MUG)) in Substrate Buffer (100 mM sodium phosphate pH 7.3, 1 mM MgCl2). The production of a fluorescent product (4-methylubelliferone) from 4-MUG was then measured using a fluorimeter at excitation wavelength 364 nm, emission at 448 nm.

Method 2

The assay is similar to that of method 1 except that the TrkAIg1,2 domain was plated directly onto the 96 well plate in Coat I Buffer and left overnight at 4°C. The wells were then emptied and Coat II Buffer added for 2 hours at 4°C. A standard curve of βNGF (0-200 nM) was preincubated for 10 minutes at room temperature with 2 µM TrkAIg1 or 2 µM TrkAIg2 and added to the plate. This was incubated at room temperature for one hour before washing and the addition of anti βNGF galactosidase conjugate. The plate was then incubated for 2 hours at room temperature and washed with Wash Buffer before adding substrate (200 mM of 4-MUG). The production of a fluorescent product was then measured using a fluorimeter at an excitation wavelength of 364 nm, emission at 448 nm..

The TrkAIg1 had no effect on NGF binding to the anti-βNGF antibodies on the plate indicating that they were not sequestering NGF in the pre-incubation. By contrast the TrkAIg2 bound to 22% of the NGF at 0.5 nM and 38% at 1 nM NGF (Figure 11)

TrkAIg2 was able to sequester NGF and thus less NGF was available for binding to the TrkAIg1,2. The binding was lowered by 40% at 200 nM NGF. TrkAIg1 was not able to sequester NGF and therefore the binding to TrkAIg1,2 was unaffected (Figure 12).

These results show that TrkAIg2 will bind to NGF resulting in a lowering of NGF concentration available for binding to a 96 well plate. TrkAIg1 is not able to do this. The

preceding protocols describe a choice of methods whereby high throughput screening of non-peptide or peptide databases may be carried out on a 96 well plate format. Competition by unknown ligands with NGF for binding to plated TrkAIg-like domains may be measured by diminution of fluorescence.

In Vitro Effects of TrkAIg-like Domains on NGF-Induced Neurite Outgrowth By PC12 Cells

PC12 (derived from a transplantable rat adrenal phaeochromocytoma, ECACC No. 88022401) cells grown in the presence of 4 ng NGF (Fig. 13A) differentiate and produce neurites after 72 hrs. This does not occur in the absence of NGF (Fig. 13B). TrkAIg2 added to PC12 cells in the presence of 4 ng NGF at 2.5 μM (Fig. 13C), 1.25 μM (Fig. 13D) and 0.625 μM (Fig. 13E) inhibits neurite outgrowth. Only when the TrkAIg2 concentration is reduced to 0.312 μM (Fig. 13F) does neurite outgrowth start to appear.

Results show that the TrkAIg2 domain is able to inhibit neurite outgrowth of PC12 cells by sequestration of NGF (Fig. 13) whereas TrkAIg1 is not able to do this.

In Vivo Effects of TrkAIg-like domains: Inhibition of Plasma Extravasation

Inhibition of NGF activity *in vivo*

All *in vivo* experiments were carried out according to the Animals (Scientific Procedures) Act 1986 under terminal anaesthesia. Plasma protein extravasation in rat skin induced by intradermal (i.d.) NGF was measured by the extravascular accumulation of intravenous (i.v.) ^{125}I-human serum albumin (Brain, S A and Williams T. J. (1985) British Journal of Pharmacology **86**: 855-860) Male Wistar rats (200-350 g) were anaesthetised with 60 mg/kg intra peritoneal (i.p.) with maintenance doses (15 mg/ml) as necessary. The dorsal skin was shaved and marked out for injection of test substances according to a balanced, randomized plan with two sites per test agent. The rats received ^{125}I-human serum albumin (100 kBq) and Evans Blue dye (0.2-0.5 ml of 2.5 % w/v in saline) i.v. via the tail vein at the start of the accumulation period. NGF and other test agents (in Tyrodes buffered salt

solution) were then injected i.d. and accumulation allowed over a 30 min period. A blood sample was taken by cardiac puncture (for plasma) and the rats killed by cervical dislocation. The dorsal skin was then removed and injection sites punched out (16 mm diameter). Plasma and skin sites were counted in a gamma counter. The plasma protein extravasation at each site was expressed as volume of plasma extravasated.

For co-injection experiments, all skin sites received 100 µl (i.d.) of either NGF (8 pmol) or Tyrode (with or without TrkAIg1,2, TrkAIg1 or TrkIg2). For pretreatment experiments, skin sites received 100 µl (i.d.) of either TrkAIg1,2 TrkAIg1 or TrkIg2 (24 or 80 pmol) or vehicle (Tyrode solution) at -5 or -40 min. These sites then received 50 µl (i.d.) NGF (8 pmol) or Tyrode at start of accumulation period (0 min).

The effect of TrkAIg1,2 on NGF-induced plasma extravasation.

The effect of co-injection of TrkAIg1,2 on NGF-induced plasma extravasation is shown in Fig. 14. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 6. The response induced by 7S NGF(7S NGF is a complex of 2.55 (β-NGF) and γ NGF), both alone and with co-injection of TrkAIg1,2, is shown (8 pmol, filled squares). For comparison, the response induced by Tyrode's solution (vehicle, open circles), alone and with co-injection of TrkAIg1,2 is also shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg1,2 differing significantly from the sites receiving agent alone are shown as ** $p < 0.01$, as assessed by ANOVA with Bonferroni's post-test.

The TrkAIg1,2 can antagonize the actions of NGF when used at a dose of 24 pmol, i.e. threefold higher than the dose of NGF used. In contrast, injection of TrkAIg1,2 in vehicle produced no significant plasma extravasation. Thus, TrkAIg1,2 can antagonize the action of NGF particularly when premixed and co-injected. This indicates that TrkAIg12 is able to bind to, and thus sequester, NGF thus inhibiting its action of extravasation. To investigate the ability of TrkAIg1,2 to antagonize NGF *in vivo*, skin sites were pre-treated by intradermal injection of TrkAIg1,2, and NGF was given (i.d.) 5 min later. The results, shown in Fig. 15, show that 24 pmol TrkAIg1,2 can significantly inhibit the plasma extravasation induced by 8 pmol 7S NGF. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 4. The response

induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and in sites pre-treated with increasing doses of TrkAIg1,2, shown. For comparison, the response induced 7S NGF (8 pmol) co-injected with TrkAIg1,2 (24 pmol) is shown in the filled bar. Plasma extravasation induced by intradermal injection of GR 73632 (30 pmol) is shown in the filled triangles and Tyrode's solution (vehicle) in the open circles, with the pre-treatment dose of TrkAIg1,2 shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg1,2 differing significantly from the sites receiving agent alone are shown as ** $p < 0.01$, as assessed by ANOVA with Bonferroni's post-test.

The plasma extravasation seen with NGF in sites pre-treated with 24 pmol TrkAIg1,2 was similar to the plasma extravasation produced by NGF co-injected with 24 pmol TrkAIg1,2. As with the co-injection experiments, pre-treatment with TrkAIg1,2 produced no significant plasma extravasation when injected alone. In an attempt to determine if the action of TrkAIg1,2 was specific to NGF-induced responses or a general anti-inflammatory effect, the NK1 agonist GR73632 (30 pmol) was injected into TrkAIg1,2 pre-treated sites. The 5 min. pre-treatment failed to inhibit the plasma extravasation induced by GR73632, as also shown in Fig 15.

In order to evaluate the stability of the NGF sequestration, skin sites were pre-treated for a longer period (40 min) with TrkAIg1,2 and NGF given (i.d.) at the start of the accumulation period, as shown in Fig. 16. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 4. The response induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and in sites pre-treated with increasing doses of TrkAIg1,2, is shown. For comparison, the response induced 7S NGF (8 pmol) co-injected with TrkAIg1,2 (24 pmol) is shown by the filled bar. Plasma extravasation induced by intradermal injection of GR73632 (30 pmol) is shown in the filled triangles and Tyrode's solution (vehicle) in the open circles, with the pre-treatment dose of TrkAIg1,2 shown on the y-axis. Plasma extravasation in sites receiving agent plus co-injected TrkAIg1,2 differing significantly from the sites receiving agent alone are shown as * $p < 0.05$, as assessed by ANOVA with Bonferroni's post-test.

In these experiments, NGF-induced plasma extravasation was significantly inhibited by 80 pmol, but not 24 pmol, TrkAIg1,2. The plasma extravasation induced by co-injection of 8

pmol NGF with 80 pmol TrkAIg1,2 is shown for comparison. In keeping with the results of the previous experiments, the doses of TrkAIg1,2 used failed to produce significant plasma extravasation when injected alone and also failed to inhibit the plasma extravasation induced by GR73632 (as before).

The effect of TrkAIg1 on NGF-induced plasma extravasation.

Following the previous series of experiments, using both immunoglobulin-like domains (TrkAIg1,2), we attempted to further characterize the binding of NGF to the immunoglobulin-like domains of TrkA. To do this, we used a sample of recombinant TrkAIg1, the first immunoglobulin-like domain. As can be seen in Fig. 17, co-injection experiments with TrkAIg1 showed no significant inhibition of NGF-induced plasma extravasation at doses up to 80 pmol/site.

Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 6. The response induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and with co-injection of TrkAIg1, shown. For comparison, the response induced by Tyrode's solution (vehicle) is shown in the open circles, with the dose of TrkAIg1 co-injected shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg1 differing significantly from the sites receiving agent alone are shown as ns, not significant, as assessed by ANOVA with Bonferroni's post-test.

The effect of TrkAIg2 on NGF-induced plasma extravasation.

The ability of TrkAIg2 to bind and sequester NGF was evaluated.

As can be seen in Fig. 18, co-injection of TrkAIg2 with NGF was able to produce significant inhibition of NGF-induced plasma extravasation, when given in a ten-fold excess. At all of the doses used, TrkAIg2 produced no inhibition of plasma extravasation induced by GR73632, and also produced no significant plasma extravasation when injected alone. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 4 - 8. The response induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and with co-injection of TrkAIg2, shown. For comparison, the response induced by GR73632 (30 pmol) is shown in the filled triangles and that induced by Tyrode's solution (vehicle) is shown in the open circles, with

the dose of TrkAIg2 co-injected shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg2 differing significantly from the sites receiving agent alone are shown as *** $p < 0.001$, as assessed by ANOVA with Bonferroni's post-test.

Pre-treatment of skin sites with 80 pmol TrkAIg2 with NGF was also able to inhibit the plasma extravasation induced by 8 pmol NGF, given 5 min later Fig. 19. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 4. The response induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and in sites pre-treated with increasing doses of TrkAIg2, shown. Plasma extravasation induced by intradermal injection of GR73632 (30 pmol) is shown in the filled triangles and Tyrode's solution (vehicle) in the open circles, with the pre-treatment dose of TrkAIg2 shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg2 differing significantly from the sites receiving agent alone are shown as [***]$p <$ 0.001, as assessed by ANOVA with Bonferroni's post-test. Again, this pre-treatment had no effect on GR73632-induced plasma extravasation, and produced no significant plasma extravasation when injected alone (Fig. 19).

Similar results were seen when TrkAIg2 was used as a 40 min pre-treatment, as shown in Fig. 20. Results are expressed as plasma extravasated (µl/site) in response to intradermal test agent, mean ± s.e.mean, n = 3. The response induced by 7S NGF (8 pmol) is shown in the filled squares, both alone and in sites pre-treated with increasing doses of TrkAIg2, shown. Plasma extravasation induced by intradermal injection of GR73632 (30 pmol) is shown in the filled triangles and Tyrode's solution (vehicle) in the open circles, with the pre-treatment dose of TrkAIg2 shown. Plasma extravasation in sites receiving agent plus co-injected TrkAIg2 differing significantly from the sites receiving agent alone are shown as *** $p < 0.001$, as assessed by ANOVA with Student-Newman-Keuls post-test. The plasma extravasation induced by NGF was significantly inhibited by TrkAIg2 at 80 pmol. For comparison, the plasma extravasation induced by 8 pmol 7S NGF co-injected with 80 pmol TrkAIg2 is shown in the filled column. Pre-treatment with TrkAIg2 induced no plasma extravasation alone and did not affect the plasma extravasation induced by GR 73632.

The results clearly demonstrate that the TrkIg2 domain is able to bind to NGF *in vivo* and block its biological activity.

Crystallisation of TrkAIg2

Crystals of recombinant TrkA-Ig2 have been obtained under a variety of conditions between 14-20% MPD, pH5.0 (100mM Na-citrate), 300 to 500mM NaCl, pH 5.0 (100mM Na-citrate), most favourably at 500mM NaCl, pH 5.0. The crystals grow reproducibly to approximate dimensions of 0.2 x 0.2 x 0.2 mm. Crystals are then cryo-preserved. Using the home source (rotating anode, mirrors, imaging plate), and the synchrotron source at Hamburg, these crystals diffract to about 2.8 Å. Assuming 50% solvent, it is estimated that there are 4 (or possibly 3) molecules in the asymmetric unit. Crystals of a selenoMet form of the protein have been prepared using a selenoMet auxotroph (there are 4 methionines in the construct) which has been used for MAD phasing and as a heavy atom derivative. Recombinant forms of both the native and selenoMet TrkA-Ig2 were prepared, purified and refolded using the established procedures as defined elsewhere in the description.

Therapeutic Aspects of TrkAIg2

Since certain pain states are caused by overexpression of NGF, it is anticipated and evidence indicates, that application of NGF antagonists such as antibodies or recombinant TrkAIg2 binding domain may alleviate resulting pain states (McMahon, S. B.*Series B-Biological Sciences*, (1996), **351**, No.1338, 431- 440; Woolf, C. J. *et al. British Journal Of Pharmacology*, (1997), **121**, **No.3**, 417- 424; Lowe, E. M. *et al. British Journal Of Urology*, (1997), **79**, **No.4**, 572-577; Dmitrieva, N. *et al. Neuroscience*, (1997), **78**, **No.2**, 449-459; Aloe, L. *et al. International Journal Of Tissue Reactions-Experimental And Clinical Aspects*, (1993), **15**, **No.4**, 139-143; Aloe, L. *et al. Rheumatology International*, (1995), **14**, **No.6**, 249-252).

Therefore, in summary, the inventors have demonstrated the inability of the region referred to as TrkAIg1 to bind NGF. The smallness of the TrkAIg2 molecule and the abundance with which this protein can be produced for example in *E. coli*, and purified and refolded into its correct formation confers certain advantages over the complete extracellular domain which, by necessity, must be made in mammalian or insect cells.

There are known to be various pain states, often chronic inflammatory conditions which are associated with an increase in NGF protein levels. These include idiopathic sensory urgency and interstitial cystitis, arthritis and shingles. It is also suggested that such chronic conditions may result in sensitization of peripheral neurons and perhaps even long-term sensory neuronal abnormalities. By sequestration of this increased NGF, by the use of TrkAIg2, it will be possible to alleviate pain in such conditions and in other conditions in which NGF is elevated.

Throughout the specification, the following abbreviations have been used:

Abbreviations for amino acids

Amino acid	*Three-letter abbreviation*	*One-letter symbol*
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Abbreviations for nucleotides:

A	Adenine
G	Guanine
C	Cytosine
T	Thymine
U	Uracil

Abbreviations for mutations:

X_1NNNX_2

X_1 and X_2 = an amino acid one letter symbol as defined above.

NNN = numerical digits indicating the position of the mutation within the amino acid sequence.

CLAIMS

1. A polypeptide consisting of or comprising the amino acid sequence of residues 22 to 119 of Fig. 4B or a portion of the amino acid sequence of Fig. 4B, the amino acid sequence being capable of binding a neurotrophin.

2. A polypeptide according to claim 1 comprising residues 22 to 144 of Fig. 4 B.

3. A polypeptide according to claim 1 or 2 wherein the polypeptide is TrkAIg1,2, or a portion thereof.

4. A polypeptide according to any one of claims 1 to 3 which binds with high affinity to a neurotrophin.

5. A polypeptide according to claim 4 which binds to a neurotrophin with a disassociation constant of less than 10nM.

6. A polypeptide according to any preceding claim wherein the polypeptide is isolated from animal cells.

7. A polypeptide according to claim 6 wherein the animal cells are mammalian cells.

8. A polypeptide according to claim 7 wherein the mammalian cells are human cells.

9. A polypeptide according to claim 6 wherein the animal cells are insect cells reptilian cells, fish cells, avian cells or amphibian cells.

10. A polypeptide according to any preceding claim wherein the neurotrophin is NGF, NT-3 or a neurotrophin which binds p75NGFR.

11. A polypeptide according to any preceding claim wherein the neurotrophin exists as a monomer, dimer, trimer, or a neurotrophin heterodimer.

12. A polypeptide according to any preceding claim wherein the neurotrophin is from a mammal, insect, reptile, fish, bird or amphibian.

13. A polypeptide according to claim 12 wherein the mammalian neurotrophin is a human neurotrophin.

14. A DNA sequence which encodes a polypeptide according to any of claims 1 to 13 or variants of such a DNA sequence due to the degeneracy of the genetic code, or insertion or deletion mutants thereof that encode a polypeptide according to any of claims 1 to 13 and DNA sequences which hybridise at 50°C, 6xSSC salt concentration to such DNA sequences.

15. A DNA sequence which encodes a polypeptide according to any of claims 1 to 13 or variants of such a DNA sequence due to the degeneracy of the genetic code, or insertion or deletion mutants thereof that encode a polypeptide according to any of claims 1 to 13 and DNA sequences which hybridise at 65°C, 2xSSC salt concentration to such DNA sequences.

16. A plasmid or other vector comprising a DNA sequence according to claim 14 or claim 15.

17. A plasmid according to claim 16 wherein the plasmid is an expression vector.

18. A plasmid according to claim 16 or claim 17 wherein the plasmid is pET-15b.

19. A complex comprising at least one polypeptide according to any of claims 1 to 13 and at least one neurotrophin or neurotrophin subunit, manomer or biologically active portion thereof.

20. A method of producing a polypeptide according to any one of claims 1 to 13 comprising introducing a DNA sequence according to claim 14 or a plasmid according to any of claims 15 to 17 into a suitable host whereby the DNA sequence is expressed.

21. A method according to claim 20 wherein the host is an animal cell.

22. A method according to claim 21 wherein the host is a bacterial cell.

23. A method according to claim 22 wherein the host is a mammalian cell.

24. A method according to claim 23 wherein the host is a human cell.

25. A method of screening for molecules which bind to the TrkA receptor using a polypeptide according to any of claims 1 to 13.

26. A method according to claim 25 comprising comparing the binding of a putative ligand to TrkAIg1, or a portion thereof, with the binding of the same putative ligand to TrkAIg2 or a portion thereof.

27. A method according to claim 25 or claim 26 comprising selecting molecules which bind to at least one solvent-exposed loop of TrkAIg2.

28. A method according to claim 27 wherein the solvent-exposed loop is loop E to F as shown in Fig.1(B).

29. A method according to claim 27 or 28 wherein the solvent-exposed loop is loop C" to D as shown in Fig.1(B).

30. A method according to claim 28 or claim 29 wherein molecules with an affinity of at least 10nM are selected.

31. A method according to any claims 25 to 30 comprising selecting molecules which enhance binding of a polypeptide according to any one of claims 1 to 13 or TrkA or a portion thereof in its natural state to a neurotrophin.

32. A method of combinatorial chemistry comprising:

1. a compound generating step

2. a compound screening step which involves the binding of the compound generated during step 1 with a polypeptide or a portion of a polypeptide according to any of claims 1 to 13.

33. An antibody raised against a polypeptide according to any of claims 1 to 13.

34. An antibody according to claim 33 wherein the polypeptide is TrkAIg2.

35. A host cell containing a DNA sequence according to claim 14 or a plasmid or other vector according to any of claims 16 to 18.

36. A host cell according to claim 35 wherein the host cell is a mammalian, bacterial, insect, or yeast cell.

37. A host cell according to claim 32 wherein the mammalian cell is a human cell.

38. A diagnostic probe wherein the probe comprises any portion of a polypeptide according to any of claims 1 to 13.

39. A diagnostic probe according to claim 38 wherein the probe is labelled.

40. A diagnostic probe according to claim 39 wherein the label comprises a fluorescent tag or a radiolabel.

41. Diagnostic tests, assays or monitoring methods using a polypeptide or any fragment of a polypeptide according to any of claims 1 to 13, or an antibody according to claim 33 or 34.

42. Diagnostic tests, assays or monitoring methods using a probe comprising at least a portion of a DNA sequence according to claim 14, or a probe according to any of claims 38 to 40.

43. Diagnostic tests, assays or monitoring methods according to claim 41 or claim 42 wherein the tests, assays, or monitoring methods comprise microbiological, animal cell, or biodiagnostic tests, assays or monitoring methods.

44. Diagnostic tests, assays or monitoring methods according to any of claims 41 to 43 which detect elevated neurotrophin levels associated with peripheral inflammation, chronic inflammation, postherpetic neuralgia, interstitial cystitis, arthritis or shingles.

45. A method of producing a polypeptide according to any of claims 1 to 13 by chemical or biological means.

46. An organism engineered to contain, express or overexpress a polypeptide according to any of claims 1 to 13 or a DNA sequence according to claim 14 or claim 15.

47. An organism according to claim 46 wherein the organism is an animal, bacteria, yeast, or insect.

48. An organism according to claim 47 wherein the animal is a mammal, bacteria, yeast or insect.

49. A composition for the control of pain associated with an increase in neurotrophin levels comprising a polypeptide according to any of claims 1 to 13.

50. A method of treating a subject with pain associated with increased neurotrophin levels, the method comprising supplying to the subject a pharmaceutical composition comprising a polypeptide according to any of claims 1 to 13 or a neurotrophin analogue isolated or identified by a screening procedure involving a polypeptide according to any of claims 1 to 13.

51. A method according to claim 50 wherein the pain is a symptom of conditions selected from idiopathic sensory urgency (ISU), interstitial cystitis, arthritis, shingles, peripheral inflammation, chronic inflammation, or postherpetic neuralgia.

52. A method of treating a subject with Alzheimers disease, the method comprising supplying to the subject a pharmaceutical composition comprising a polypeptide according to any of claims 1 to 13.

53. A method of treating a subject with Alzheimers disease, the method comprising supplying to the subject a pharmaceutical composition comprising an neurotrophin analogue isolated or identified by a screening procedure involving a polypeptide according to any of claims 1 to 13.

54. A method of reducing free NGF levels in a subject, the method comprising supplying to a subject, a polypeptide according to any of claims 1 to 13.

55. A method of reducing plasma extravasation comprising supplying to a subject, a polypeptide according to any of claims 1 to 13.

56. A method according to any of claims 50 to 555 in which the neurotrophin is NGF.

57. A pharmaceutical composition comprising a polypeptide according to any of claims 1 to 13 together with a pharmaceutically acceptable carrier or diluent.

58. A pharmaceutical composition according to claim 57 including at least one neurotrophin.

59. A machine readable data storage medium, comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using the data, is capable of displaying a graphical three-dimensional representation of a polypeptide according to any of claims 1 to 13.

60. A homology model having the coordinates shown in Fig. 21.

61. A computer programmed with or arranged to provide a homology model for at least a portion of a polypeptide according to any one of claims 1 to 13, or a complex of such a polypetide with another molecule.

62. A machine readable data storage medium on which has been stored in machine readable form a homology model of a polypeptide according to any one of claims 1 to 13 or a complex of such a polypetide with another molecule.

63. A computer according to claim 61 or a machine readable data storage medium according to claim 62 in which the model is obtained from coordinates shown in Fig. 21.

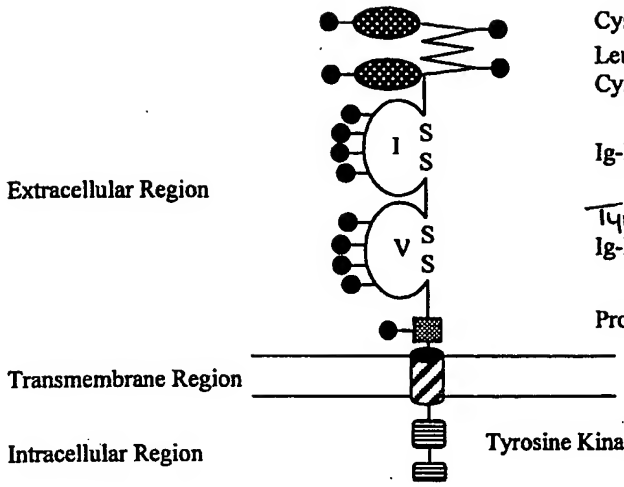
64. Compounds obtained by a method according to any of claims 25 to 32 or using a computer according to claim 61 or 63 or using a machine readable data storage medium according to claim 62 or 63.

65. Crystalline Trk AIg2.

66. A crystal comprising at least a portion of a polypeptide according to any of claims 1 to 11.

67. A crystal according to claim 63 wherein a polypeptide is TrkAIg2.

Fig. 1(A).



	Residues
Cysteine cluster 1	1-35
Leucine rich motif	36-107
Cysteine cluster 2	108-159
Ig-like sub-domain1	160-252
Tyrosine kinase domain	
Ig-like sub-domain2	253-349
Proline Rich region	350-375

Fig. 1(B)

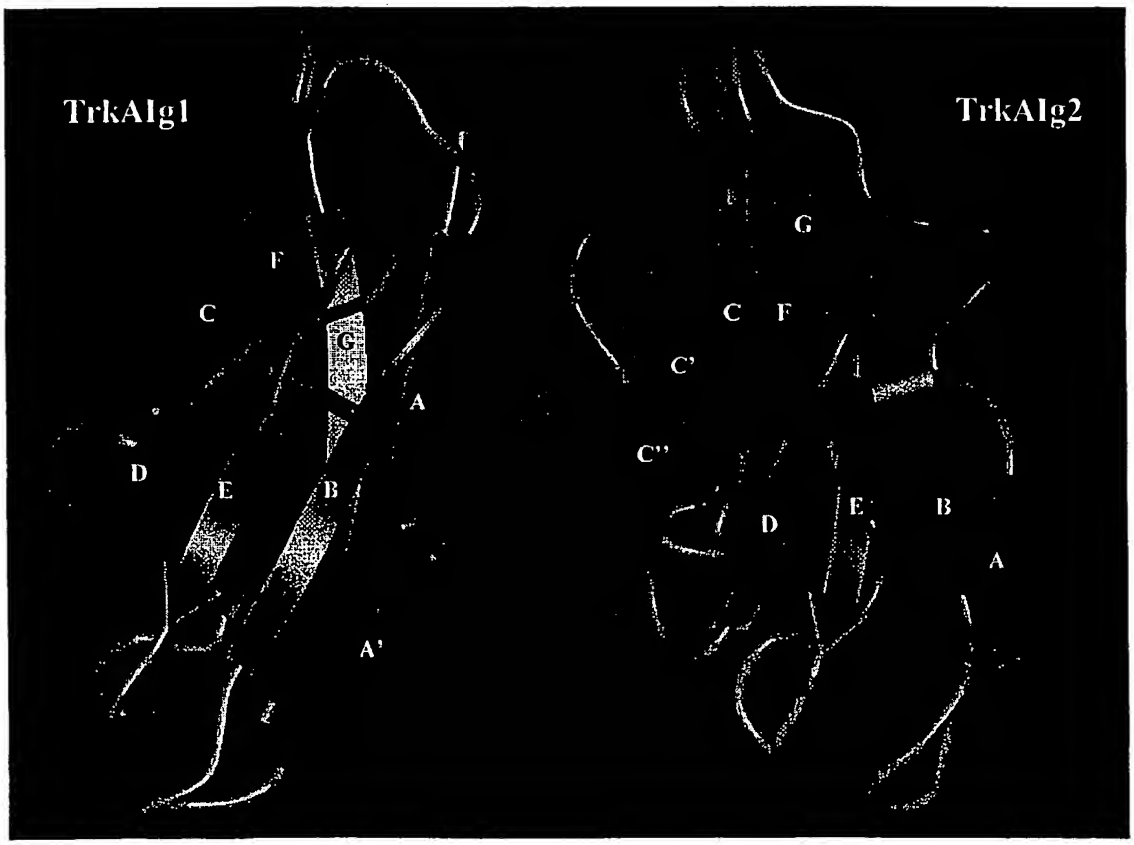
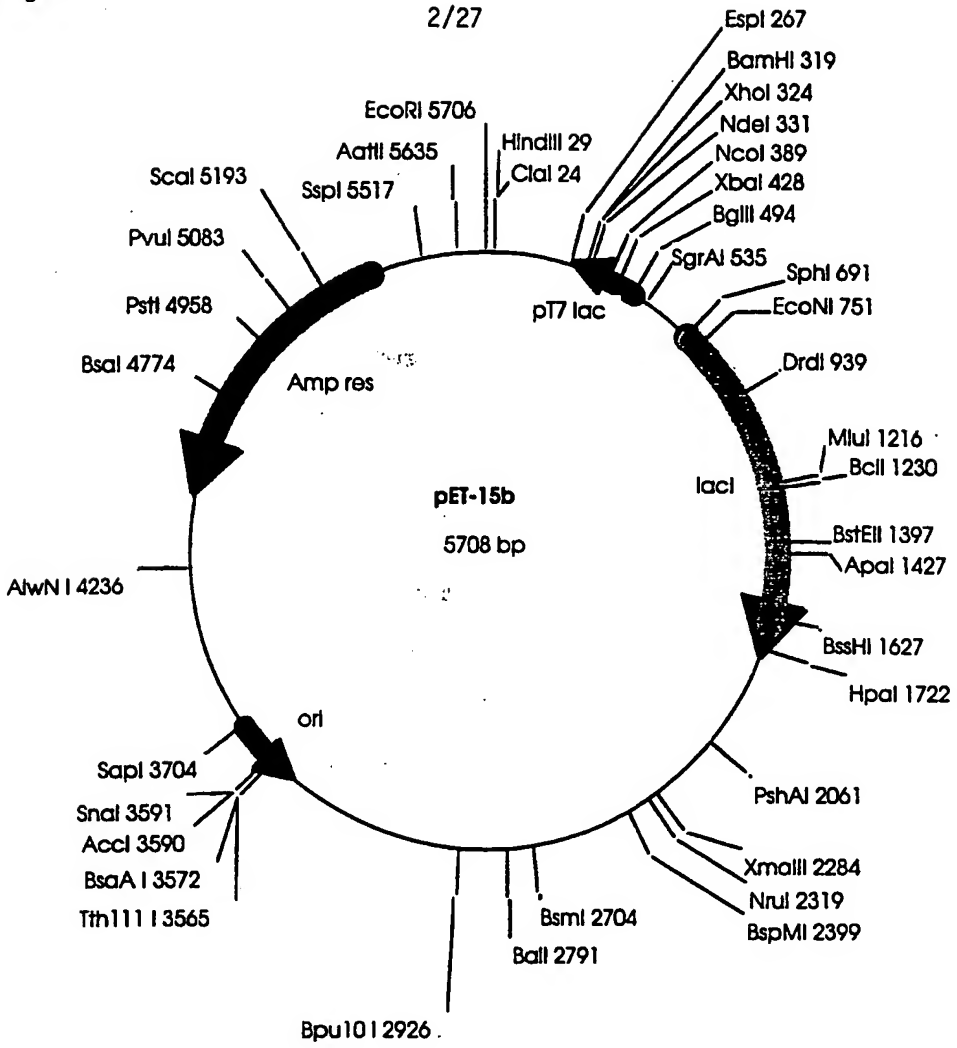


Fig. 2.



A)

B)

Oligo10692 Left Primer for TrkAIg1,2

CCGATCTCGAGGGTGTGCCCACGCTG

XhoI

Oligo10693 Right Primer for TrkAIg1,2

CCGATCTCGAG TTA TCA TTCGTCCTTCTTCTCCACCGGGTC

XhoI Stop Stop

Fig. 3.


```
     Met Gly Ser Ser His His His His His His Ser Ser     12
   1 ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC
     Gly Leu Val Pro Arg Gly Ser His Met Leu Glu Gly     24
  38 GGC CTG GTG CCG CGC GGC AGC CAT ATG CTC GAG GGT
     Val Pro Thr Leu Lys Val Gln Val Pro Asn Ala Ser     36
  74 GTG CCC ACG CTG AAG GTC CAG GTG CCC AAT GCC TCG
     Val Asp Val Gly Asp Asp Val Leu Leu Arg Cys Gln     48
 110 GTG GAT GTG GGG GAC GAC GTG CTG CTG CGG TGC CAG
     Val Glu Gly Arg Gly Leu Glu Gln Ala Gly Trp Ile     60
 146 GTG GAG GGG CGG GGC CTG GAG CAG GCC GGC TGG ATC
     Leu Thr Glu Leu Glu Gln Ser Ala Thr Val Met Lys     72
 182 CTC ACA GAG CTG GAG CAG TCA GCC ACG GTG ATG AAA
     Ser Gly Gly Leu Pro Ser Leu Gly Leu Thr Leu Ala     84
 218 TCT GGG GGT CTG CCA TCC CTG GGG CTG ACC CTG GCC
     Asn Val Thr Ser Asp Leu Asn Arg Lys Asn Leu Thr     96
 254 AAT GTC ACC AGT GAC CTC AAC AGG AAG AAC TTG ACG
     Cys Trp Ala Glu Asn Asp Val Gly Arg Ala Glu Val    108
 290 TGC TGG GCA GAG AAC GAT GTG GGC CGG GCA GAG GTC
     Ser Val Gln Val Asn Val Ser Phe Pro Ala Ser Val    120
 326 TCT GTT CAG GTC AAC GTC TCC TTC CCG GCC AGT GTG
     Gln Leu His Thr Ala Val Glu Met His His Trp Cys    132
 362 CAG CTG CAC ACG GCG GTG GAG ATG CAC CAC TGG TGC
     Ile Pro Phe Ser Val Asp Gly Gln Pro Ala Pro Ser    144
 398 ATC CCC TTC TCT GTG GAT GGG CAG CCG GCA CCG TCT
     Leu Arg Trp Leu Phe Asn Gly Ser Val Leu Asn Glu    156
 434 CTG CGC TGG CTC TTC AAT GGC TCC GTG CTC AAT GAG
     Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro Ala    168
 470 ACC AGC TTC ATC TTC ACT GAG TTC CTG GAG CCG GCA
     Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg Leu    180
 506 GCC AAT GAG ACC GTG CGG CAC GGG TGT CTG CGC CTC
     Asn Gln Pro Thr His Val Asn Asn Gly Asn Tyr Thr    192
 542 AAC CAG CCC ACC CAC GTC AAC AAC GGC AAC TAC ACG
     Leu Leu Ala Ala Asn Pro Phe Gly Gln Ala Ser Ala    204
 578 CTG CTG GCT GCC AAC CCC TTC GGC CAG GCC TCC GCC
     Ser Ile Met Ala Ala Phe Met Asp Asn Pro Phe Glu    216
 614 TCC ATC ATG GCT GCC TTC ATG GAC AAC CCT TTC GAG
     Phe Asn Pro Glu Asp Pro Ile Pro Asp Thr Asn Ser    228
 650 TTC AAC CCC GAG GAC CCC ATC CCT GAC ACT AAC AGC
     Thr Ser Gly Asp Pro Val Glu Lys Lys Asp Glu Stop   239
 686 ACA TCT GGA GAC CCG GTG GAG AAG AAG GAC GAA TGA
 722 TAACTCGAGATCGG
```

Fig.4.

A)

```
     Met Gly Ser Ser His His His His His His Ser Ser      12
   1 ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC
     Gly Leu Val Pro Arg Gly Ser His Met Leu Glu Gly      24
  38 GGC CTG GTG CCG CGC GGC AGC CAT ATG CTC GAG GGT
     Val Pro Thr Leu Lys Val Gln Val Pro Asn Ala Ser      36
  74 GTG CCC ACG CTG AAG GTC CAG GTG CCC AAT GCC TCG
     Val Asp Val Gly Asp Asp Val Leu Leu Arg Cys Gln      48
 110 GTG GAT GTG GGG GAC GAC GTG CTG CTG CGG TGC CAG
     Val Glu Gly Arg Gly Leu Glu Gln Ala Gly Trp Ile      60
 146 GTG GAG GGG CGG GGC CTG GAG CAG GCC GGC TGG ATC
     Leu Thr Glu Leu Glu Gln Ser Ala Thr Val Met Lys      72
 182 CTC ACA GAG CTG GAG CAG TCA GCC ACG GTG ATG AAA
     Ser Gly Gly Leu Pro Ser Leu Gly Leu Thr Leu Ala      84
 218 TCT GGG GGT CTG CCA TCC CTG GGG CTG ACC CTG GCC
     Asn Val Thr Ser Asp Leu Asn Arg Lys Asn Leu Thr      96
 254 AAT GTC ACC AGT GAC CTC AAC AGG AAG AAC TTG ACG
     Cys Trp Ala Glu Asn Asp Val Gly Arg Ala Glu Val     108
 290 TGC TGG GCA GAG AAC GAT GTG GGC CGG GCA GAG GTC
     Ser Val Gln Val Asn Val Ser Phe Stop                116
 326 TCT GTT CAG GTC AAC GTC TCC TTC TGA TAACTCGAGCG
```

B)

```
     Met Gly Ser Ser His His His His His His Ser Ser      12
   1 ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC AGC
     Gly Leu Val Pro Arg Gly Ser His Met Pro Ala Ser      24
  38 GGC CTG GTG CCG CGC GGC AGC CAT ATG CCG GCC AGT
     Val Gln Leu His Thr Ala Val Glu Met His His Trp      36
  74 GTG CAG CTG CAC ACG GCG GTG GAG ATG CAC CAC TGG
     Cys Ile Pro Phe Ser Val Asp Gly Gln Pro Ala Pro      48
 110 TGC ATC CCC TTC TCT GTG GAT GGG CAG CCG GCA CCG
     Ser Leu Arg Trp Leu Phe Asn Gly Ser Val Leu Asn      60
 146 TCT CTG CGC TGG CTC TTC AAT GGC TCC GTG CTC AAT
     Glu Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro      72
 182 GAG ACC AGC TTC ATC TTC ACT GAG TTC CTG GAG CCG
     Ala Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg      84
 218 GCA GCC AAT GAG ACC GTG CGG CAC GGG TGT CTG CGC
     Leu Asn Gln Pro Thr His Val Asn Asn Gly Asn Tyr      96
 254 CTC AAC CAG CCC ACC CAC GTC AAC AAC GGC AAC TAC
     Thr Leu Leu Ala Ala Asn Pro Phe Gly Gln Ala Ser     108
 290 ACG CTG CTG GCT GCC AAC CCC TTC GGC CAG GCC TCC
     Ala Ser Ile Met Ala Ala Phe Met Asp Asn Pro Phe     120
 326 GCC TCC ATC ATG GCT GCC TTC ATG GAC AAC CCT TTC
     Glu Phe Asn Pro Glu Asp Pro Ile Pro Asp Thr Asn     132
 362 GAG TTC AAC CCC GAG GAC CCC ATC CCT GAC ACT AAC
     Ser Thr Ser Gly Asp Pro Val Glu Lys Lys Asp Glu     144
 398 AGC ACA TCT GGA GAC CCG GTG GAG AAG AAG GAC GAA
     Stop                                                144
 434 TGA TAACTCGAGATCGG
```

C)


```
     Met Gly Ser Ser His His His His His His Ser Ser      12
   1 ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC
     Gly Leu Val Pro Arg Gly Ser His Met Pro Ala Ser      24
  38 GGC CTG GTG CCG CGC GGC AGC CAT ATG CCG GCC AGT
     Val Gln Leu His Thr Ala Val Glu Met His His Trp      36
  74 GTG CAG CTG CAC ACG GCG GTG GAG ATG CAC CAC TGG
     Ser Ile Pro Phe Ser Val Asp Gly Gln Pro Ala Pro      48
 110 TCG ATC CCC TTC TCT GTG GAT GGG CAG CCG GCA CCG
     Ser Leu Arg Trp Leu Phe Asn Gly Ser Val Leu Asn      60
 146 TCT CTG CGC TGG CTC TTC AAT GGC TCC GTG CTC AAT
     Glu Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro      72
 182 GAG ACC AGC TTC ATC TTC ACT GAG TTC CTG GAG CCG
     Ala Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg      84
 218 GCA GCC AAT GAG ACC GTG CGG CAC GGG TGT CTG CGC
     Leu Asn Gln Pro Thr His Val Asn Asn Gly Asn Tyr      96
 254 CTC AAC CAG CCC ACC CAC GTC AAC AAC GGC AAC TAC
     Thr Leu Leu Ala Ala Asn Pro Phe Gly Gln Ala Ser     108
 290 ACG CTG CTG GCT GCC AAC CCC TTC GGC CAG GCC TCC
     Ala Ser Ile Met Ala Ala Phe Met Asp Asn Pro Phe     120
 326 GCC TCC ATC ATG GCT GCC TTC ATG GAC AAC CCT TTC
     Glu Phe Asn Pro Glu Asp Pro Ile Pro Val Ser Phe     132
 362 GAG TTC AAC CCC GAG GAC CCC ATC CCT GTC TCC TTC
     Ser Pro Val Asp Thr Asn Ser Thr Ser Gly Asp Pro     144
 398 TCG CCA GTG GAC ACT AAC AGC ACA TCT GGA GAC CCG
     Val Glu Lys Lys Asp Glu Stop                        150
 434 GTG GAG AAG AAG GAC GAA TGA TAACTCGAGATCGG
```

Fig. 5.

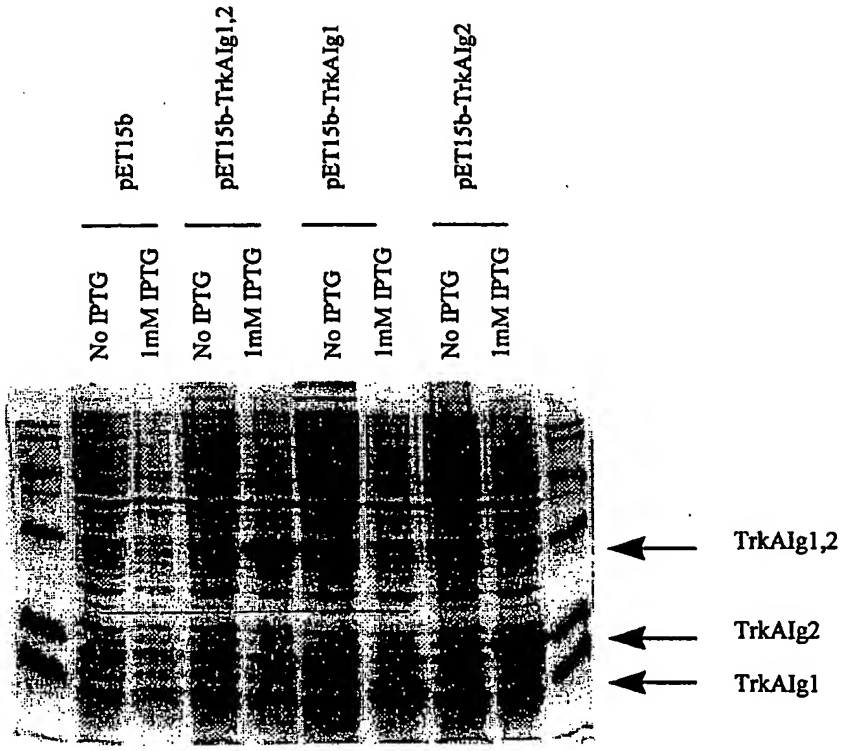


Fig. 6A.

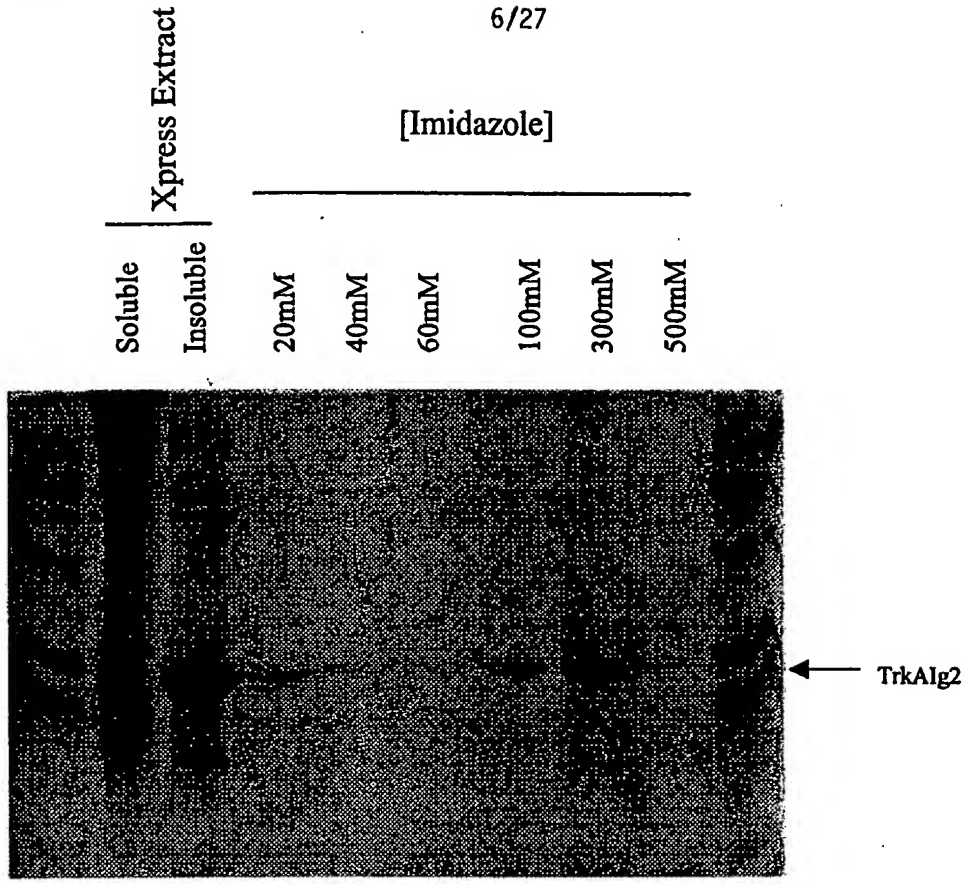

Xpress Extract
[Imidazole]
Soluble
Insoluble
20mM
40mM
60mM
100mM
300mM
500mM
TrkAIg2

Fig. 6B.

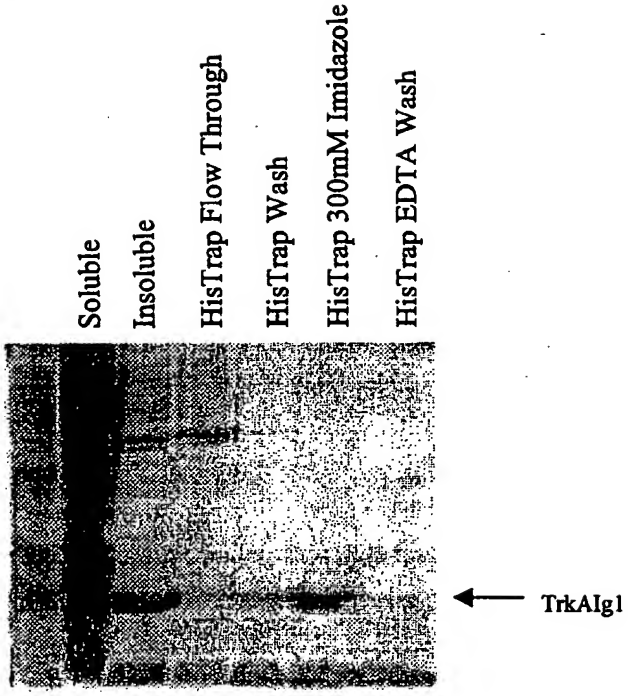
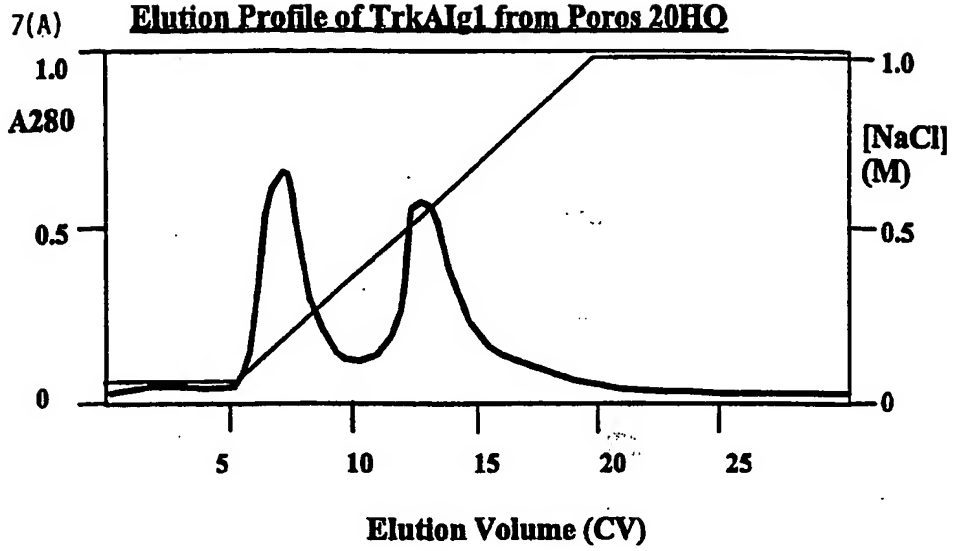

Soluble
Insoluble
HisTrap Flow Through
HisTrap Wash
HisTrap 300mM Imidazole
HisTrap EDTA Wash
TrkAIg1

Fig. 7(A)


Elution Profile of TrkAIg1 from Poros 20HQ
1.0
A280
0.5
0
1.0
[NaCl]
(M)
0.5
0
5
10
15
20
25
Elution Volume (CV)

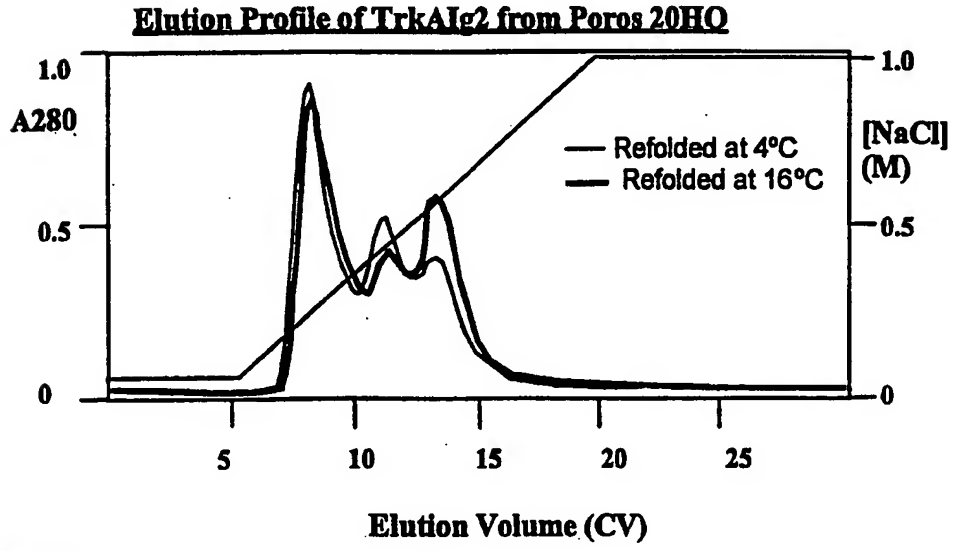

Elution Profile of TrkAIg2 from Poros 20HQ
1.0
A280
0.5
0
Refolded at 4°C
Refolded at 16°C
1.0
[NaCl]
(M)
0.5
0
5
10
15
20
25
Elution Volume (CV)

Fig. 7(B)

Fig. 8

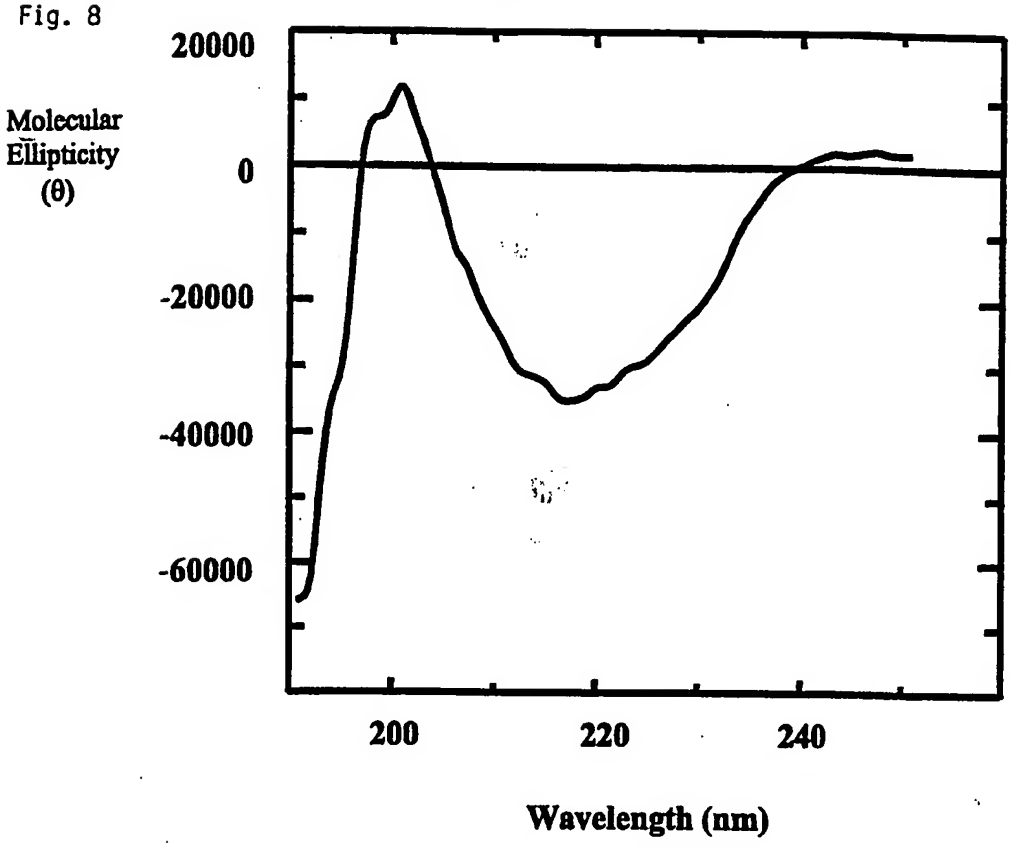

20000
0
-20000
-40000
-60000
Molecular Ellipticity (θ)
200
220
240
Wavelength (nm)

Fig. 9.

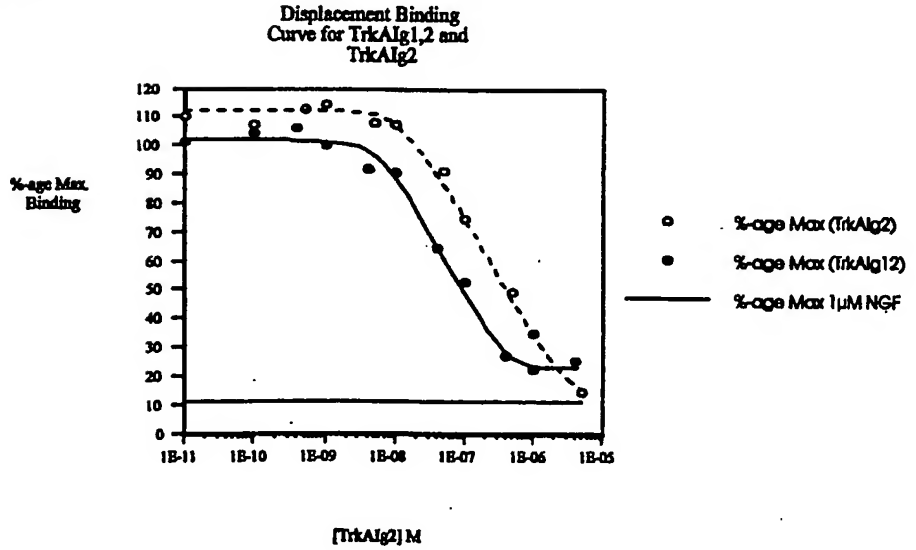

Displacement Binding Curve for TrkAIg1,2 and TrkAIg2
%-age Max. Binding
120
110
100
90
80
70
60
50
40
30
20
10
0
1E-11
1E-10
1E-09
1E-08
1E-07
1E-06
1E-05
[TrkAIg2] M
%-age Max (TrkAIg2)
%-age Max (TrkAIg12)
%-age Max 1μM NGF

Fig. 10.

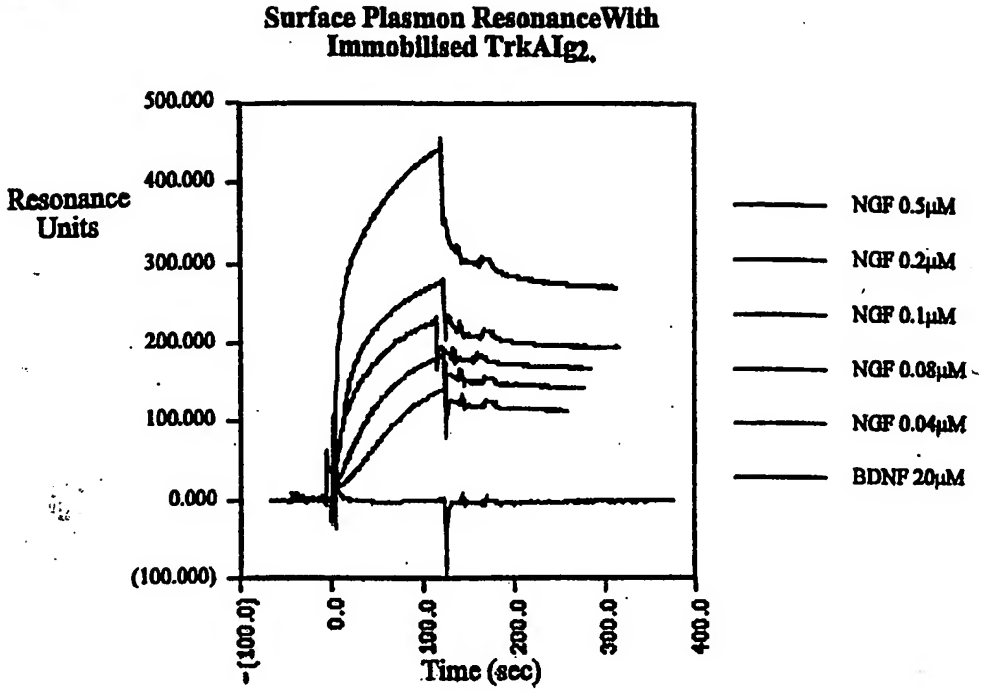

Surface Plasmon ResonanceWith Immobilised TrkAIg2.
Resonance Units
500.000
400.000
300.000
200.000
100.000
0.000
(100.000)
-100.0
0.0
100.0
200.0
300.0
400.0
Time (sec)
NGF 0.5μM
NGF 0.2μM
NGF 0.1μM
NGF 0.08μM
NGF 0.04μM
BDNF 20μM

Fig. 11.

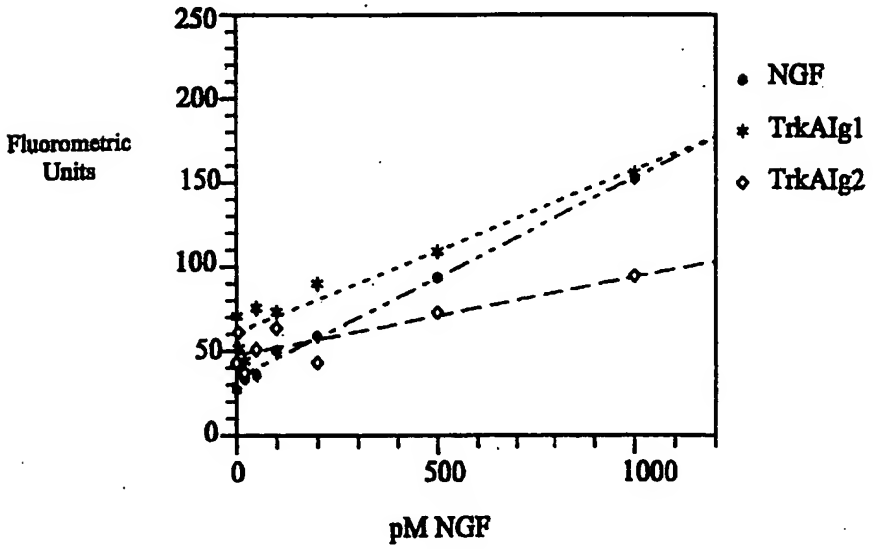

Fluorometric Units
250
200
150
100
50
0
0
500
1000
pM NGF
NGF
TrkAIg1
TrkAIg2

Fig. 12.

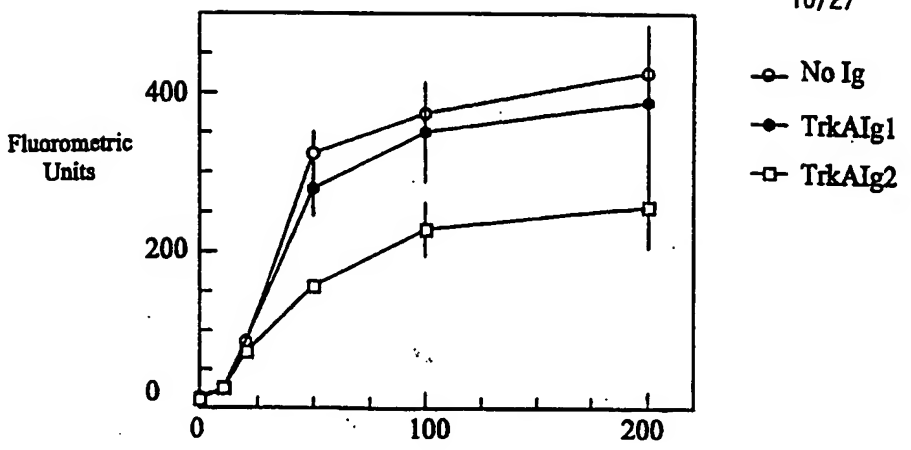

10/27
Fluorometric Units
400
200
0
0
100
200
NGF nM
No Ig
TrkAIg1
TrkAIg2

Fig. 13.

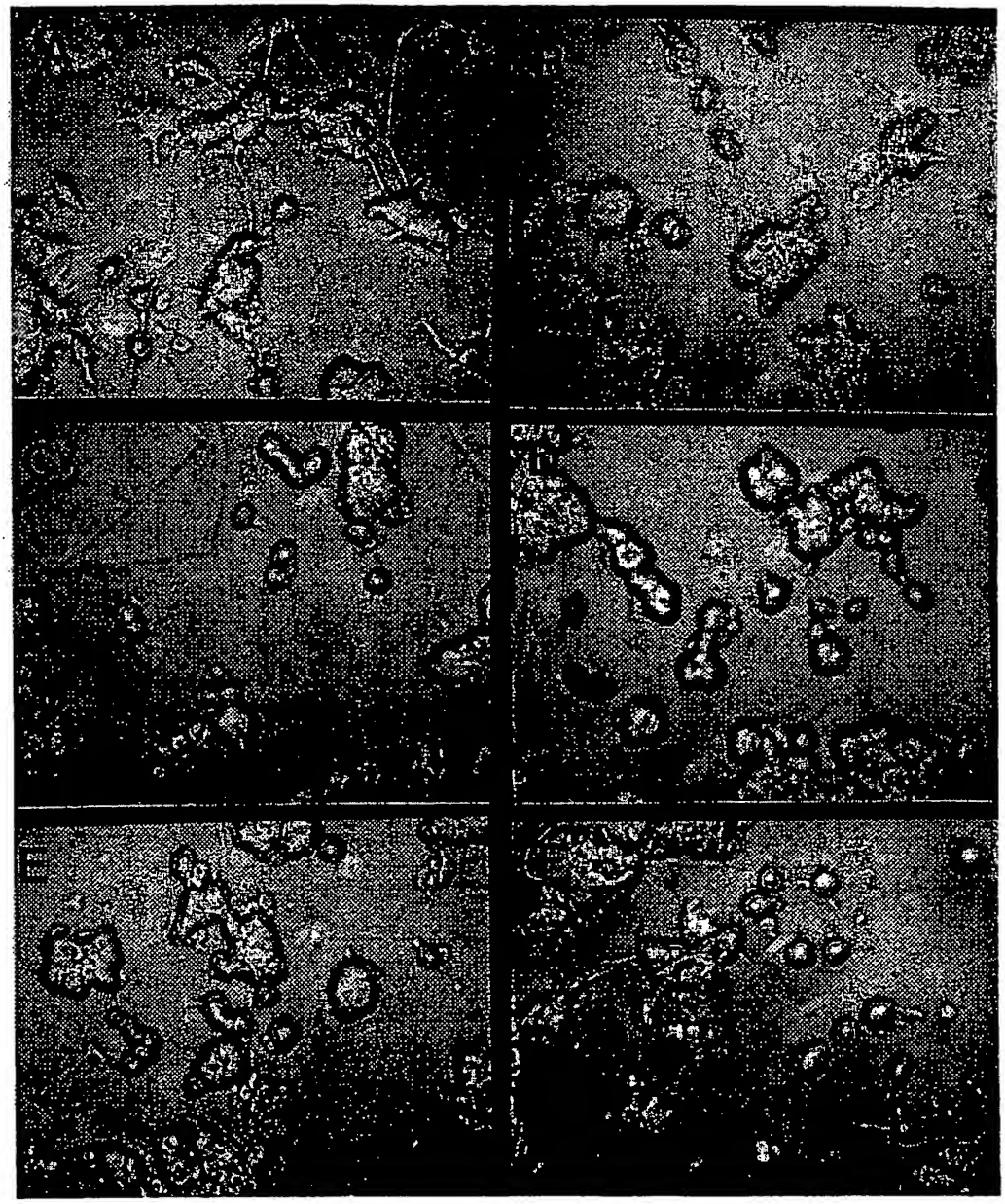
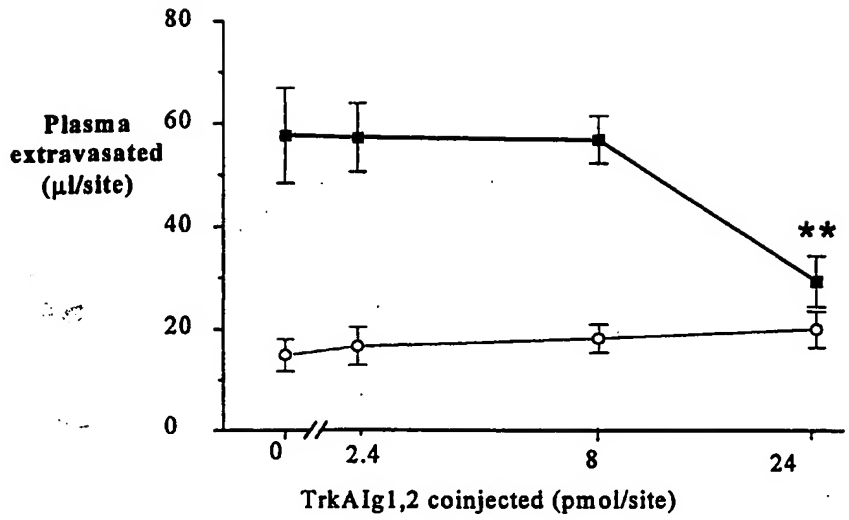


Fig. 14.


80
60
40
20
0
Plasma extravasated (µl/site)
**
0
2.4
8
24
TrkAIg1,2 coinjected (pmol/site)

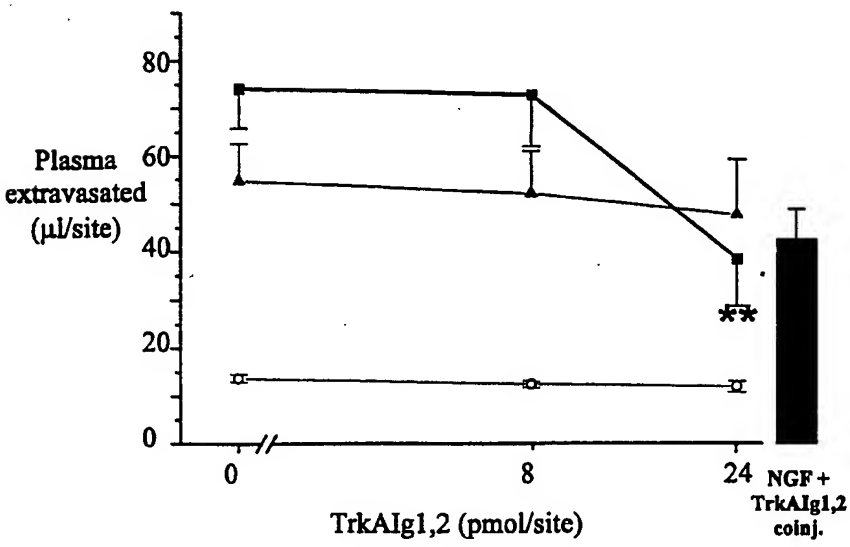

80
60
40
20
0
Plasma extravasated (µl/site)
**
0
8
24
NGF + TrkAIg1,2 coinj.
TrkAIg1,2 (pmol/site)

Fig. 15.

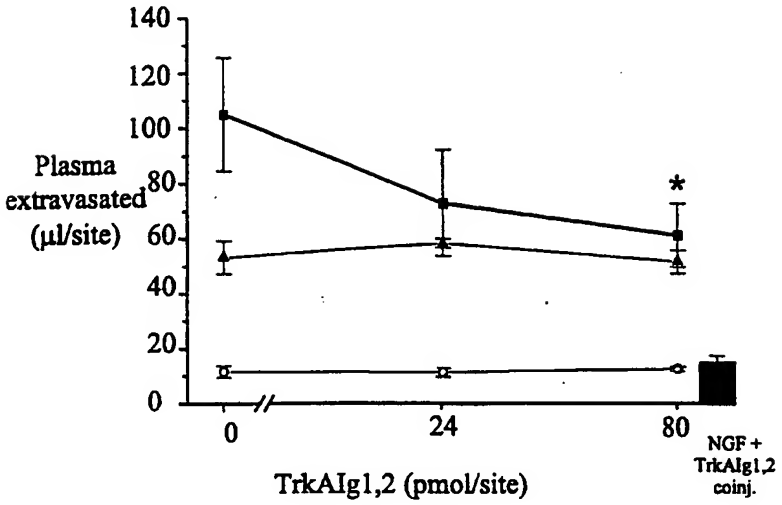

140
120
100
80
60
40
20
0
Plasma extravasated (µl/site)
*
0
24
80
NGF + TrkAIg1,2 coinj.
TrkAIg1,2 (pmol/site)

Fig. 16.

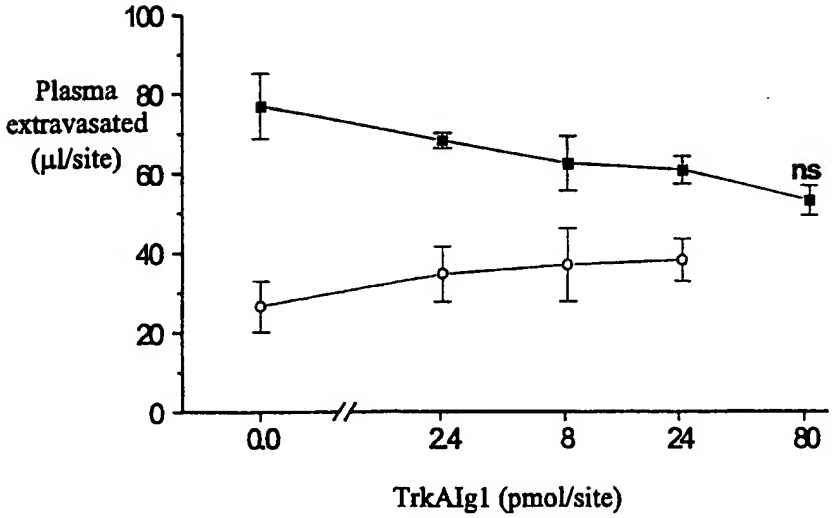

100
80
60
40
20
0
Plasma extravasated (µl/site)
ns
0.0
24
8
24
80
TrkAIg1 (pmol/site)

Fig. 17.

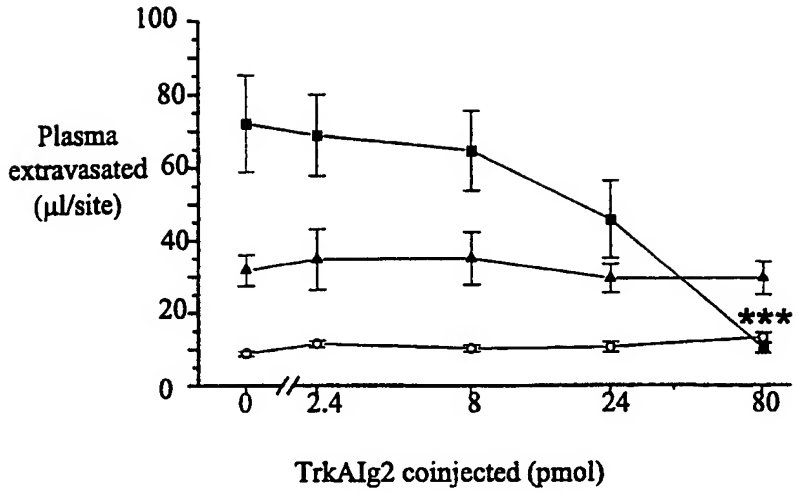

100
80
60
40
20
0
Plasma extravasated (µl/site)
***
0
2.4
8
24
80
TrkAIg2 coinjected (pmol)

Fig. 18.

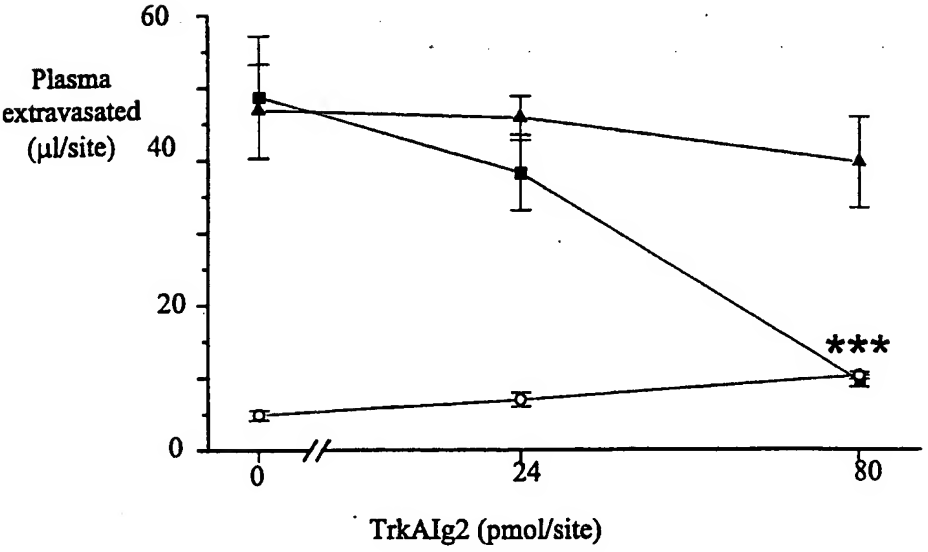

60
40
20
0
Plasma extravasated (µl/site)
***
0
24
80
TrkAIg2 (pmol/site)

Fig. 19

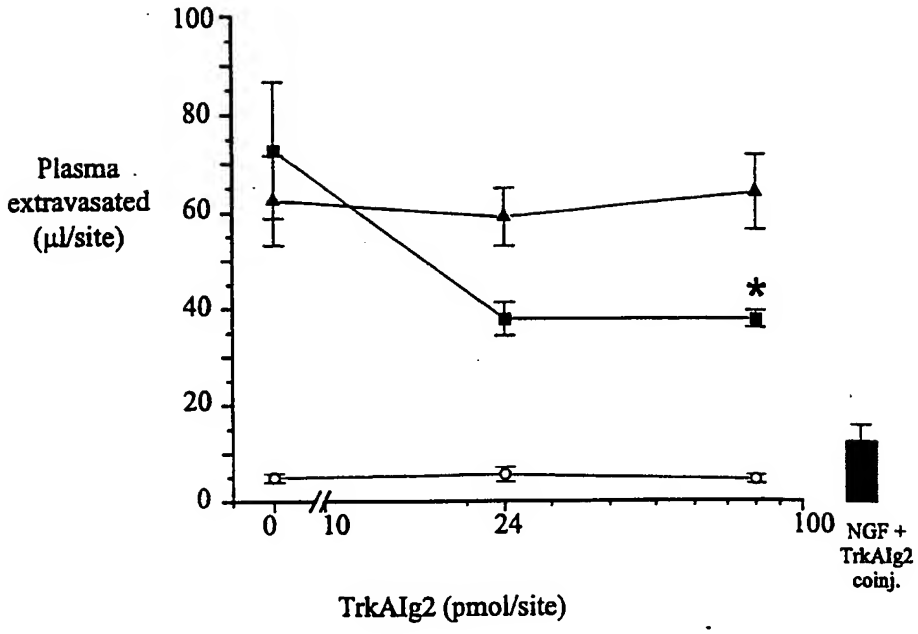

Plasma extravasated (µl/site)
100
80
60
40
20
0
0
10
24
100
*
NGF + TrkAIg2 coinj.
TrkAIg2 (pmol/site)

Fig. 20.

Fig. 21


```
REMARK   TrkA domain 2
REMARK
SHEET    1 SEX   SER S   3  LEU S   6
SHEET    2 SEX   HIS S  13  CYS S  16
SHEET    3 SEX   SER S  28  LEU S  32
SHEET    4 SEX   LEU S  38  SER S  42
SHEET    5 SEX   GLU S  47  GLU S  50
SHEET    6 SEX   ALA S  53  VAL S  57
SHEET    7 SEX   CYS S  61  ASN S  65
SHEET    8 SEX   THR S  76  ASN S  81
SHEET    9 SEX   GLY S  84  ALA S  88
TURN     1      PRO S   7  ALA S  11
TURN     2      SER S  17  VAL S  20
TURN     3      LEU S  35  LEU S  37
TURN     4      LEU S  42  HIS S  45
TURN     5      HIS S  51  HIS S  52
TURN     6      THR S  58  THR S  59
TURN     7      MET S  82  MET S  83
SSBOND   1 CYS S   16     CYS S   61
ATOM      1  N   PRO S   1      25.261 138.102  16.261  1.00  0.00
ATOM      2  CA  PRO S   1      25.908 137.182  17.231  1.00  0.00
ATOM      3  C   PRO S   1      27.226 137.778  17.735  1.00  0.00
ATOM      4  O   PRO S   1      27.661 138.818  17.283  1.00  0.00
ATOM      5  CB  PRO S   1      26.158 135.916  16.416  1.00  0.00
ATOM      6  CG  PRO S   1      26.226 136.374  14.994  1.00  0.00
ATOM      7  CD  PRO S   1      25.365 137.605  14.880  1.00  0.00
ATOM      8  N   ALA S   2      27.863 137.129  18.673  1.00  0.00
ATOM      9  CA  ALA S   2      29.147 137.662  19.204  1.00  0.00
ATOM     10  C   ALA S   2      30.334 136.946  18.552  1.00  0.00
ATOM     11  O   ALA S   2      30.246 136.466  17.439  1.00  0.00
ATOM     12  CB  ALA S   2      29.116 137.510  20.735  1.00  0.00
ATOM     13  N   SER S   3      31.442 136.873  19.236  1.00  0.00
ATOM     14  CA  SER S   3      32.633 136.190  18.656  1.00  0.00
ATOM     15  C   SER S   3      33.500 135.589  19.764  1.00  0.00
ATOM     16  O   SER S   3      33.256 135.791  20.937  1.00  0.00
ATOM     17  CB  SER S   3      33.393 137.278  17.924  1.00  0.00
ATOM     18  OG  SER S   3      33.868 138.236  18.860  1.00  0.00
ATOM     19  N   VAL S   4      34.518 134.856  19.396  1.00  0.00
ATOM     20  CA  VAL S   4      35.419 134.237  20.420  1.00  0.00
ATOM     21  C   VAL S   4      36.421 133.276  19.783  1.00  0.00
ATOM     22  O   VAL S   4      36.512 133.123  18.577  1.00  0.00
ATOM     23  CB  VAL S   4      34.556 133.490  21.513  1.00  0.00
ATOM     24  CG1 VAL S   4      33.872 132.169  21.068  1.00  0.00
ATOM     25  CG2 VAL S   4      35.345 133.129  22.795  1.00  0.00
ATOM     26  N   GLN S   5      37.157 132.611  20.620  1.00  0.00
ATOM     27  CA  GLN S   5      38.157 131.621  20.143  1.00  0.00
ATOM     28  C   GLN S   5      38.093 130.394  21.051  1.00  0.00
ATOM     29  O   GLN S   5      38.538 130.421  22.181  1.00  0.00
ATOM     30  CB  GLN S   5      39.552 132.283  20.151  1.00  0.00
ATOM     31  CG  GLN S   5      39.779 133.429  19.110  1.00  0.00
ATOM     32  CD  GLN S   5      39.726 133.100  17.612  1.00  0.00
ATOM     33  OE1 GLN S   5      40.247 132.089  17.171  1.00  0.00
ATOM     34  NE2 GLN S   5      39.138 133.925  16.783  1.00  0.00
ATOM     35  N   LEU S   6      37.535 129.322  20.568  1.00  0.00
ATOM     36  CA  LEU S   6      37.435 128.093  21.423  1.00  0.00
ATOM     37  C   LEU S   6      38.594 127.146  21.128  1.00  0.00
ATOM     38  O   LEU S   6      39.504 127.475  20.397  1.00  0.00
ATOM     39  CB  LEU S   6      36.064 127.391  21.210  1.00  0.00
ATOM     40  CG  LEU S   6      34.840 127.921  22.004  1.00  0.00
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ATOM     41  CD1 LEU S   6      34.865 127.375  23.439  1.00  0.00
ATOM     42  CD2 LEU S   6      34.772 129.458  22.035  1.00  0.00
ATOM     43  N   HIS S   7      38.564 125.968  21.690  1.00  0.00
ATOM     44  CA  HIS S   7      39.722 125.213  21.289  1.00  0.00
ATOM     45  C   HIS S   7      39.524 123.698  21.380  1.00  0.00
ATOM     46  O   HIS S   7      38.384 123.188  21.436  1.00  0.00
ATOM     47  CB  HIS S   7      40.908 125.664  22.188  1.00  0.00
ATOM     48  CG  HIS S   7      42.119 124.819  21.872  1.00  0.00
ATOM     49  ND1 HIS S   7      42.551 124.574  20.613  1.00  0.00
ATOM     50  CD2 HIS S   7      42.953 124.134  22.769  1.00  0.00
ATOM     51  CE1 HIS S   7      43.603 123.745  20.744  1.00  0.00
ATOM     52  NE2 HIS S   7      43.868 123.468  22.021  1.00  0.00
ATOM     53  N   THR S   8      40.680 123.000  21.431  1.00  0.00
ATOM     54  CA  THR S   8      40.815 121.569  21.109  1.00  0.00
ATOM     55  C   THR S   8      39.587 120.616  21.219  1.00  0.00
ATOM     56  O   THR S   8      38.960 120.395  22.332  1.00  0.00
ATOM     57  CB  THR S   8      41.972 121.037  21.933  1.00  0.00
ATOM     58  OG1 THR S   8      42.669 120.062  21.171  1.00  0.00
ATOM     59  CG2 THR S   8      41.468 120.390  23.235  1.00  0.00
ATOM     60  N   ALA S   9      39.249 120.074  19.968  1.00  0.00
ATOM     61  CA  ALA S   9      38.454 118.829  19.787  1.00  0.00
ATOM     62  C   ALA S   9      37.251 118.752  20.710  1.00  0.00
ATOM     63  O   ALA S   9      36.995 117.716  21.365  1.00  0.00
ATOM     64  CB  ALA S   9      39.376 117.660  20.007  1.00  0.00
ATOM     65  N   VAL S  10      36.492 119.834  20.758  1.00  0.00
ATOM     66  CA  VAL S  10      36.254 120.358  22.047  1.00  0.00
ATOM     67  C   VAL S  10      34.802 120.909  22.345  1.00  0.00
ATOM     68  O   VAL S  10      33.805 120.141  22.521  1.00  0.00
ATOM     69  CB  VAL S  10      37.284 121.436  22.177  1.00  0.00
ATOM     70  CG1 VAL S  10      37.961 121.303  23.514  1.00  0.00
ATOM     71  CG2 VAL S  10      38.331 121.282  21.066  1.00  0.00
ATOM     72  N   GLU S  11      34.720 122.270  22.326  1.00  0.00
ATOM     73  CA  GLU S  11      34.480 123.086  23.593  1.00  0.00
ATOM     74  C   GLU S  11      33.044 123.702  23.901  1.00  0.00
ATOM     75  O   GLU S  11      32.262 123.138  24.739  1.00  0.00
ATOM     76  CB  GLU S  11      35.519 124.181  23.462  1.00  0.00
ATOM     77  CG  GLU S  11      35.501 125.138  24.601  1.00  0.00
ATOM     78  CD  GLU S  11      36.545 126.192  24.357  1.00  0.00
ATOM     79  OE1 GLU S  11      36.715 127.008  25.222  1.00  0.00
ATOM     80  OE2 GLU S  11      37.184 126.186  23.288  1.00  0.00
ATOM     81  N   MET S  12      32.751 124.775  23.233  1.00  0.00
ATOM     82  CA  MET S  12      31.427 125.429  23.416  1.00  0.00
ATOM     83  C   MET S  12      31.004 126.157  22.137  1.00  0.00
ATOM     84  O   MET S  12      30.315 125.609  21.300  1.00  0.00
ATOM     85  CB  MET S  12      31.557 126.421  24.555  1.00  0.00
ATOM     86  CG  MET S  12      31.870 125.730  25.889  1.00  0.00
ATOM     87  SD  MET S  12      32.043 126.899  27.244  1.00  0.00
ATOM     88  CE  MET S  12      32.387 125.712  28.551  1.00  0.00
ATOM     89  N   HIS S  13      31.408 127.382  21.980  1.00  0.00
ATOM     90  CA  HIS S  13      31.032 128.146  20.757  1.00  0.00
ATOM     91  C   HIS S  13      29.527 128.109  20.528  1.00  0.00
ATOM     92  O   HIS S  13      28.991 127.162  19.988  1.00  0.00
ATOM     93  CB  HIS S  13      31.762 127.510  19.580  1.00  0.00
ATOM     94  CG  HIS S  13      33.222 127.891  19.650  1.00  0.00
ATOM     95  ND1 HIS S  13      34.051 127.508  20.653  1.00  0.00
ATOM     96  CD2 HIS S  13      33.950 128.671  18.746  1.00  0.00
ATOM     97  CE1 HIS S  13      35.251 128.044  20.356  1.00  0.00
ATOM     98  NE2 HIS S  13      35.220 128.745  19.220  1.00  0.00
ATOM     99  N   HIS S  14      28.850 129.145  20.904  1.00  0.00
ATOM    100  CA  HIS S  14      27.382 129.193  20.683  1.00  0.00
ATOM    101  C   HIS S  14      27.016 130.561  20.107  1.00  0.00
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ATOM	102	O	HIS	S	14	27.447	131.584	20.601	1.00	0.00
ATOM	103	CB	HIS	S	14	26.730	128.971	22.058	1.00	0.00
ATOM	104	CG	HIS	S	14	27.070	127.629	22.639	1.00	0.00
ATOM	105	ND1	HIS	S	14	26.417	126.431	22.359	1.00	0.00
ATOM	106	CD2	HIS	S	14	28.105	127.445	23.544	1.00	0.00
ATOM	107	CE1	HIS	S	14	27.133	125.594	23.135	1.00	0.00
ATOM	108	NE2	HIS	S	14	28.149	126.115	23.874	1.00	0.00
ATOM	109	N	TRP	S	15	26.242	130.593	19.062	1.00	0.00
ATOM	110	CA	TRP	S	15	25.876	131.900	18.460	1.00	0.00
ATOM	111	C	TRP	S	15	24.365	131.983	18.264	1.00	0.00
ATOM	112	O	TRP	S	15	23.701	130.988	18.052	1.00	0.00
ATOM	113	CB	TRP	S	15	26.577	131.999	17.118	1.00	0.00
ATOM	114	CG	TRP	S	15	28.063	131.962	17.347	1.00	0.00
ATOM	115	CD1	TRP	S	15	28.908	133.072	17.566	1.00	0.00
ATOM	116	CD2	TRP	S	15	28.907	130.786	17.383	1.00	0.00
ATOM	117	NE1	TRP	S	15	30.206	132.710	17.743	1.00	0.00
ATOM	118	CE2	TRP	S	15	30.232	131.231	17.622	1.00	0.00
ATOM	119	CE3	TRP	S	15	28.661	129.425	17.227	1.00	0.00
ATOM	120	CZ2	TRP	S	15	31.265	130.312	17.709	1.00	0.00
ATOM	121	CZ3	TRP	S	15	29.694	128.506	17.314	1.00	0.00
ATOM	122	CH2	TRP	S	15	31.006	128.955	17.552	1.00	0.00
ATOM	123	N	CYS	S	16	23.816	133.160	18.327	1.00	0.00
ATOM	124	CA	CYS	S	16	22.457	133.496	18.183	1.00	0.00
ATOM	125	C	CYS	S	16	22.258	134.927	18.480	1.00	0.00
ATOM	126	O	CYS	S	16	22.586	135.408	19.605	1.00	0.00
ATOM	127	CB	CYS	S	16	21.703	132.638	19.173	1.00	0.00
ATOM	128	SG	CYS	S	16	19.922	132.873	19.077	1.00	0.00
ATOM	129	N	ILE	S	17	21.796	135.659	17.467	1.00	0.00
ATOM	130	CA	ILE	S	17	21.625	137.083	17.695	1.00	0.00
ATOM	131	C	ILE	S	17	20.223	137.436	18.157	1.00	0.00
ATOM	132	O	ILE	S	17	19.263	137.501	17.348	1.00	0.00
ATOM	133	CB	ILE	S	17	21.943	137.815	16.391	1.00	0.00
ATOM	134	CG1	ILE	S	17	22.054	139.327	16.656	1.00	0.00
ATOM	135	CG2	ILE	S	17	20.836	137.589	15.364	1.00	0.00
ATOM	136	CD1	ILE	S	17	22.761	140.068	15.520	1.00	0.00
ATOM	137	N	PRO	S	18	20.084	137.607	19.471	1.00	0.00
ATOM	138	CA	PRO	S	18	18.821	138.065	19.995	1.00	0.00
ATOM	139	C	PRO	S	18	18.308	139.267	19.219	1.00	0.00
ATOM	140	O	PRO	S	18	17.124	139.406	18.932	1.00	0.00
ATOM	141	CB	PRO	S	18	19.028	138.436	21.470	1.00	0.00
ATOM	142	CG	PRO	S	18	20.525	138.302	21.793	1.00	0.00
ATOM	143	CD	PRO	S	18	21.148	138.094	20.336	1.00	0.00
ATOM	144	N	PHE	S	19	19.228	140.179	18.875	1.00	0.00
ATOM	145	CA	PHE	S	19	18.797	141.326	18.074	1.00	0.00
ATOM	146	C	PHE	S	19	18.034	140.915	16.867	1.00	0.00
ATOM	147	O	PHE	S	19	17.580	141.768	16.033	1.00	0.00
ATOM	148	CB	PHE	S	19	20.025	142.135	17.663	1.00	0.00
ATOM	149	CG	PHE	S	19	19.895	143.469	18.331	1.00	0.00
ATOM	150	CD1	PHE	S	19	20.170	143.597	19.685	1.00	0.00
ATOM	151	CD2	PHE	S	19	19.393	144.542	17.612	1.00	0.00
ATOM	152	CE1	PHE	S	19	19.930	144.800	20.327	1.00	0.00
ATOM	153	CE2	PHE	S	19	19.160	145.750	18.264	1.00	0.00
ATOM	154	CZ	PHE	S	19	19.424	145.880	19.624	1.00	0.00
ATOM	155	N	SER	S	20	17.950	139.567	16.695	1.00	0.00
ATOM	156	CA	SER	S	20	16.868	138.992	15.860	1.00	0.00
ATOM	157	C	SER	S	20	17.080	139.163	14.368	1.00	0.00
ATOM	158	O	SER	S	20	17.855	140.071	13.942	1.00	0.00
ATOM	159	CB	SER	S	20	15.575	139.695	16.291	1.00	0.00
ATOM	160	OG	SER	S	20	15.837	141.102	16.423	1.00	0.00
ATOM	161	N	VAL	S	21	15.885	138.323	13.764	1.00	0.00
ATOM	162	CA	VAL	S	21	15.991	138.891	12.422	1.00	0.00

ATOM	163	C	VAL	S	21	14.596	139.156	11.835	1.00	0.00
ATOM	164	O	VAL	S	21	13.712	138.263	11.884	1.00	0.00
ATOM	165	CB	VAL	S	21	16.723	137.887	11.521	1.00	0.00
ATOM	166	CG1	VAL	S	21	16.178	136.482	11.752	1.00	0.00
ATOM	167	CG2	VAL	S	21	16.517	138.257	10.049	1.00	0.00
ATOM	168	N	ASP	S	22	14.140	140.310	11.434	1.00	0.00
ATOM	169	CA	ASP	S	22	12.707	140.438	11.055	1.00	0.00
ATOM	170	C	ASP	S	22	12.549	140.092	9.579	1.00	0.00
ATOM	171	O	ASP	S	22	13.508	140.011	8.840	1.00	0.00
ATOM	172	CB	ASP	S	22	12.329	141.897	11.323	1.00	0.00
ATOM	173	CG	ASP	S	22	12.290	142.179	12.831	1.00	0.00
ATOM	174	OD1	ASP	S	22	11.957	141.271	13.575	1.00	0.00
ATOM	175	OD2	ASP	S	22	12.595	143.297	13.212	1.00	0.00
ATOM	176	N	GLY	S	23	11.345	139.890	9.152	1.00	0.00
ATOM	177	CA	GLY	S	23	11.114	139.540	7.722	1.00	0.00
ATOM	178	C	GLY	S	23	9.731	140.014	7.282	1.00	0.00
ATOM	179	O	GLY	S	23	9.222	141.007	7.764	1.00	0.00
ATOM	180	N	GLN	S	24	9.118	139.316	6.363	1.00	0.00
ATOM	181	CA	GLN	S	24	7.769	139.736	5.890	1.00	0.00
ATOM	182	C	GLN	S	24	7.193	138.705	4.901	1.00	0.00
ATOM	183	O	GLN	S	24	7.087	138.988	3.725	1.00	0.00
ATOM	184	CB	GLN	S	24	7.885	141.130	5.236	1.00	0.00
ATOM	185	CG	GLN	S	24	6.539	141.817	4.827	1.00	0.00
ATOM	186	CD	GLN	S	24	6.577	143.203	4.172	1.00	0.00
ATOM	187	OE1	GLN	S	24	7.637	143.767	3.950	1.00	0.00
ATOM	188	NE2	GLN	S	24	5.460	143.813	3.867	1.00	0.00
ATOM	189	N	PRO	S	25	6.805	137.546	5.398	1.00	0.00
ATOM	190	CA	PRO	S	25	6.929	137.190	6.830	1.00	0.00
ATOM	191	C	PRO	S	25	8.136	136.266	7.043	1.00	0.00
ATOM	192	O	PRO	S	25	8.532	135.546	6.150	1.00	0.00
ATOM	193	CB	PRO	S	25	5.633	136.428	7.090	1.00	0.00
ATOM	194	CG	PRO	S	25	5.209	135.877	5.752	1.00	0.00
ATOM	195	CD	PRO	S	25	6.086	136.497	4.685	1.00	0.00
ATOM	196	N	ALA	S	26	8.702	136.264	8.222	1.00	0.00
ATOM	197	CA	ALA	S	26	9.862	135.375	8.510	1.00	0.00
ATOM	198	C	ALA	S	26	10.981	135.547	7.467	1.00	0.00
ATOM	199	O	ALA	S	26	10.858	135.095	6.346	1.00	0.00
ATOM	200	CB	ALA	S	26	9.336	133.931	8.577	1.00	0.00
ATOM	201	N	PRO	S	27	12.050	136.187	7.874	1.00	0.00
ATOM	202	CA	PRO	S	27	13.198	136.405	6.956	1.00	0.00
ATOM	203	C	PRO	S	27	13.952	135.095	6.713	1.00	0.00
ATOM	204	O	PRO	S	27	13.526	134.033	7.120	1.00	0.00
ATOM	205	CB	PRO	S	27	14.085	137.384	7.719	1.00	0.00
ATOM	206	CG	PRO	S	27	13.745	137.163	9.158	1.00	0.00
ATOM	207	CD	PRO	S	27	12.296	136.763	9.204	1.00	0.00
ATOM	208	N	SER	S	28	15.081	135.173	6.064	1.00	0.00
ATOM	209	CA	SER	S	28	15.887	133.951	5.801	1.00	0.00
ATOM	210	C	SER	S	28	17.330	134.208	6.234	1.00	0.00
ATOM	211	O	SER	S	28	17.789	135.332	6.234	1.00	0.00
ATOM	212	CB	SER	S	28	15.773	133.525	4.316	1.00	0.00
ATOM	213	OG	SER	S	28	16.338	134.484	3.416	1.00	0.00
ATOM	214	N	LEU	S	29	18.050	133.191	6.611	1.00	0.00
ATOM	215	CA	LEU	S	29	19.466	133.421	7.049	1.00	0.00
ATOM	216	C	LEU	S	29	20.429	132.569	6.214	1.00	0.00
ATOM	217	O	LEU	S	29	20.055	131.551	5.664	1.00	0.00
ATOM	218	CB	LEU	S	29	19.622	133.124	8.568	1.00	0.00
ATOM	219	CG	LEU	S	29	19.235	134.240	9.575	1.00	0.00
ATOM	220	CD1	LEU	S	29	20.371	135.266	9.687	1.00	0.00
ATOM	221	CD2	LEU	S	29	17.927	134.955	9.196	1.00	0.00
ATOM	222	N	ARG	S	30	21.666	132.977	6.111	1.00	0.00
ATOM	223	CA	ARG	S	30	22.641	132.189	5.308	1.00	0.00

ATOM	224	C	ARG	S	30	24.018	132.227	5.965	1.00	0.00
ATOM	225	O	ARG	S	30	24.899	132.935	5.524	1.00	0.00
ATOM	226	CB	ARG	S	30	22.676	132.754	3.862	1.00	0.00
ATOM	227	CG	ARG	S	30	23.417	131.864	2.829	1.00	0.00
ATOM	228	CD	ARG	S	30	23.445	132.487	1.429	1.00	0.00
ATOM	229	NE	ARG	S	30	24.147	131.551	0.514	1.00	0.00
ATOM	230	CZ	ARG	S	30	24.360	131.752	-0.779	1.00	0.00
ATOM	231	NH1	ARG	S	30	23.980	132.812	-1.429	1.00	0.00
ATOM	232	NH2	ARG	S	30	24.983	130.833	-1.428	1.00	0.00
ATOM	233	N	TRP	S	31	24.224	131.467	7.007	1.00	0.00
ATOM	234	CA	TRP	S	31	25.571	131.477	7.655	1.00	0.00
ATOM	235	C	TRP	S	31	26.608	131.059	6.620	1.00	0.00
ATOM	236	O	TRP	S	31	26.372	130.178	5.818	1.00	0.00
ATOM	237	CB	TRP	S	31	25.490	130.474	8.809	1.00	0.00
ATOM	238	CG	TRP	S	31	24.550	131.007	9.829	1.00	0.00
ATOM	239	CD1	TRP	S	31	23.216	130.849	9.797	1.00	0.00
ATOM	240	CD2	TRP	S	31	24.845	131.793	11.019	1.00	0.00
ATOM	241	NE1	TRP	S	31	22.661	131.495	10.886	1.00	0.00
ATOM	242	CE2	TRP	S	31	23.627	132.093	11.670	1.00	0.00
ATOM	243	CE3	TRP	S	31	26.038	132.273	11.588	1.00	0.00
ATOM	244	CZ2	TRP	S	31	23.594	132.846	12.844	1.00	0.00
ATOM	245	CZ3	TRP	S	31	26.005	133.028	12.770	1.00	0.00
ATOM	246	CH2	TRP	S	31	24.787	133.315	13.397	1.00	0.00
ATOM	247	N	LEU	S	32	27.742	131.699	6.616	1.00	0.00
ATOM	248	CA	LEU	S	32	28.784	131.367	5.611	1.00	0.00
ATOM	249	C	LEU	S	32	30.043	132.188	5.861	1.00	0.00
ATOM	250	O	LEU	S	32	29.992	133.349	6.218	1.00	0.00
ATOM	251	CB	LEU	S	32	28.152	131.687	4.232	1.00	0.00
ATOM	252	CG	LEU	S	32	27.768	133.174	4.091	1.00	0.00
ATOM	253	CD1	LEU	S	32	28.993	133.957	3.631	1.00	0.00
ATOM	254	CD2	LEU	S	32	26.664	133.348	3.045	1.00	0.00
ATOM	255	N	PHE	S	33	31.169	131.582	5.667	1.00	0.00
ATOM	256	CA	PHE	S	33	32.454	132.282	5.871	1.00	0.00
ATOM	257	C	PHE	S	33	33.583	131.325	5.513	1.00	0.00
ATOM	258	O	PHE	S	33	34.361	130.920	6.353	1.00	0.00
ATOM	259	CB	PHE	S	33	32.585	132.738	7.356	1.00	0.00
ATOM	260	CG	PHE	S	33	32.691	131.623	8.407	1.00	0.00
ATOM	261	CD1	PHE	S	33	33.950	131.117	8.756	1.00	0.00
ATOM	262	CD2	PHE	S	33	31.545	131.063	8.975	1.00	0.00
ATOM	263	CE1	PHE	S	33	34.058	130.055	9.645	1.00	0.00
ATOM	264	CE2	PHE	S	33	31.654	130.005	9.873	1.00	0.00
ATOM	265	CZ	PHE	S	33	32.910	129.498	10.206	1.00	0.00
ATOM	266	N	ASN	S	34	33.650	130.914	4.276	1.00	0.00
ATOM	267	CA	ASN	S	34	34.697	129.928	3.887	1.00	0.00
ATOM	268	C	ASN	S	34	34.514	128.668	4.739	1.00	0.00
ATOM	269	O	ASN	S	34	35.424	127.883	4.918	1.00	0.00
ATOM	270	CB	ASN	S	34	36.106	130.563	4.077	1.00	0.00
ATOM	271	CG	ASN	S	34	36.518	131.648	3.075	1.00	0.00
ATOM	272	OD1	ASN	S	34	36.324	131.533	1.874	1.00	0.00
ATOM	273	ND2	ASN	S	34	37.115	132.721	3.520	1.00	0.00
ATOM	274	N	GLY	S	35	33.328	128.478	5.271	1.00	0.00
ATOM	275	CA	GLY	S	35	33.056	127.286	6.120	1.00	0.00
ATOM	276	C	GLY	S	35	31.881	126.501	5.536	1.00	0.00
ATOM	277	O	GLY	S	35	31.116	125.886	6.251	1.00	0.00
ATOM	278	N	SER	S	36	31.723	126.528	4.239	1.00	0.00
ATOM	279	CA	SER	S	36	30.588	125.793	3.607	1.00	0.00
ATOM	280	C	SER	S	36	29.269	126.322	4.163	1.00	0.00
ATOM	281	O	SER	S	36	28.500	125.596	4.760	1.00	0.00
ATOM	282	CB	SER	S	36	30.744	124.264	3.804	1.00	0.00
ATOM	283	OG	SER	S	36	31.915	123.737	3.169	1.00	0.00
ATOM	284	N	VAL	S	37	29.020	127.595	3.969	1.00	0.00

ATOM	285	CA	VAL	S	37	27.811	128.253	4.425	1.00	0.00
ATOM	286	C	VAL	S	37	27.367	127.743	5.797	1.00	0.00
ATOM	287	O	VAL	S	37	26.149	127.679	6.103	1.00	0.00
ATOM	288	CB	VAL	S	37	26.714	127.993	3.378	1.00	0.00
ATOM	289	CG1	VAL	S	37	25.359	128.461	3.900	1.00	0.00
ATOM	290	CG2	VAL	S	37	27.032	128.743	2.086	1.00	0.00
ATOM	291	N	LEU	S	38	28.366	127.332	6.620	1.00	0.00
ATOM	292	CA	LEU	S	38	28.052	126.711	7.913	1.00	0.00
ATOM	293	C	LEU	S	38	26.753	125.947	7.891	1.00	0.00
ATOM	294	O	LEU	S	38	26.705	124.711	8.147	1.00	0.00
ATOM	295	CB	LEU	S	38	27.925	127.809	8.970	1.00	0.00
ATOM	296	CG	LEU	S	38	29.126	128.735	9.025	1.00	0.00
ATOM	297	CD1	LEU	S	38	28.777	130.105	9.613	1.00	0.00
ATOM	298	CD2	LEU	S	38	30.247	128.170	9.896	1.00	0.00
ATOM	299	N	ASN	S	39	25.675	126.692	7.632	1.00	0.00
ATOM	300	CA	ASN	S	39	24.357	126.130	7.806	1.00	0.00
ATOM	301	C	ASN	S	39	23.279	126.989	7.185	1.00	0.00
ATOM	302	O	ASN	S	39	23.465	128.203	6.943	1.00	0.00
ATOM	303	CB	ASN	S	39	24.112	126.005	9.301	1.00	0.00
ATOM	304	CG	ASN	S	39	22.686	125.595	9.516	1.00	0.00
ATOM	305	OD1	ASN	S	39	22.347	124.420	9.586	1.00	0.00
ATOM	306	ND2	ASN	S	39	21.824	126.622	9.609	1.00	0.00
ATOM	307	N	GLU	S	40	22.111	126.468	6.900	1.00	0.00
ATOM	308	CA	GLU	S	40	21.022	127.276	6.398	1.00	0.00
ATOM	309	C	GLU	S	40	19.698	126.735	6.922	1.00	0.00
ATOM	310	O	GLU	S	40	19.501	125.521	6.855	1.00	0.00
ATOM	311	CB	GLU	S	40	20.992	127.237	4.894	1.00	0.00
ATOM	312	CG	GLU	S	40	19.960	128.182	4.298	1.00	0.00
ATOM	313	CD	GLU	S	40	19.931	128.272	2.794	1.00	0.00
ATOM	314	OE1	GLU	S	40	19.218	129.105	2.210	1.00	0.00
ATOM	315	OE2	GLU	S	40	20.658	127.492	2.172	1.00	0.00
ATOM	316	N	THR	S	41	18.841	127.619	7.441	1.00	0.00
ATOM	317	CA	THR	S	41	17.464	127.340	7.853	1.00	0.00
ATOM	318	C	THR	S	41	16.612	127.970	6.761	1.00	0.00
ATOM	319	O	THR	S	41	16.859	129.136	6.461	1.00	0.00
ATOM	320	CB	THR	S	41	17.194	127.906	9.288	1.00	0.00
ATOM	321	OG1	THR	S	41	18.058	127.294	10.236	1.00	0.00
ATOM	322	CG2	THR	S	41	15.776	127.683	9.857	1.00	0.00
ATOM	323	N	SER	S	42	15.627	127.286	6.201	1.00	0.00
ATOM	324	CA	SER	S	42	14.747	127.887	5.217	1.00	0.00
ATOM	325	C	SER	S	42	13.377	127.367	5.584	1.00	0.00
ATOM	326	O	SER	S	42	13.266	126.188	5.873	1.00	0.00
ATOM	327	CB	SER	S	42	15.220	127.555	3.779	1.00	0.00
ATOM	328	OG	SER	S	42	16.514	128.089	3.481	1.00	0.00
ATOM	329	N	PHE	S	43	12.369	128.209	5.531	1.00	0.00
ATOM	330	CA	PHE	S	43	11.048	127.921	6.037	1.00	0.00
ATOM	331	C	PHE	S	43	10.341	126.626	5.679	1.00	0.00
ATOM	332	O	PHE	S	43	10.229	125.847	6.621	1.00	0.00
ATOM	333	CB	PHE	S	43	10.046	129.023	5.575	1.00	0.00
ATOM	334	CG	PHE	S	43	8.606	128.898	6.093	1.00	0.00
ATOM	335	CD1	PHE	S	43	8.324	129.188	7.433	1.00	0.00
ATOM	336	CD2	PHE	S	43	7.583	128.438	5.261	1.00	0.00
ATOM	337	CE1	PHE	S	43	7.041	129.003	7.937	1.00	0.00
ATOM	338	CE2	PHE	S	43	6.297	128.263	5.763	1.00	0.00
ATOM	339	CZ	PHE	S	43	6.027	128.542	7.102	1.00	0.00
ATOM	340	N	ILE	S	44	9.835	126.212	4.530	1.00	0.00
ATOM	341	CA	ILE	S	44	9.139	124.919	4.536	1.00	0.00
ATOM	342	C	ILE	S	44	10.107	123.838	4.134	1.00	0.00
ATOM	343	O	ILE	S	44	9.842	123.092	3.206	1.00	0.00
ATOM	344	CB	ILE	S	44	7.844	124.957	3.627	1.00	0.00
ATOM	345	CG1	ILE	S	44	8.119	125.214	2.111	1.00	0.00

ATOM	346	CG2	ILE	S	44	6.794	126.003	4.113	1.00	0.00
ATOM	347	CD1	ILE	S	44	6.959	124.882	1.149	1.00	0.00
ATOM	348	N	PHE	S	45	11.226	123.703	4.810	1.00	0.00
ATOM	349	CA	PHE	S	45	12.233	122.765	4.373	1.00	0.00
ATOM	350	C	PHE	S	45	13.025	122.195	5.507	1.00	0.00
ATOM	351	O	PHE	S	45	12.819	122.593	6.650	1.00	0.00
ATOM	352	CB	PHE	S	45	13.228	123.464	3.397	1.00	0.00
ATOM	353	CG	PHE	S	45	12.619	124.092	2.134	1.00	0.00
ATOM	354	CD1	PHE	S	45	12.159	125.413	2.170	1.00	0.00
ATOM	355	CD2	PHE	S	45	12.462	123.341	0.966	1.00	0.00
ATOM	356	CE1	PHE	S	45	11.533	125.970	1.060	1.00	0.00
ATOM	357	CE2	PHE	S	45	11.845	123.902	-0.148	1.00	0.00
ATOM	358	CZ	PHE	S	45	11.378	125.215	-0.101	1.00	0.00
ATOM	359	N	THR	S	46	13.898	121.240	5.218	1.00	0.00
ATOM	360	CA	THR	S	46	14.725	120.690	6.282	1.00	0.00
ATOM	361	C	THR	S	46	16.076	121.403	6.180	1.00	0.00
ATOM	362	O	THR	S	46	16.491	121.794	5.089	1.00	0.00
ATOM	363	CB	THR	S	46	14.833	119.134	6.146	1.00	0.00
ATOM	364	OG1	THR	S	46	15.519	118.784	4.951	1.00	0.00
ATOM	365	CG2	THR	S	46	13.500	118.359	6.067	1.00	0.00
ATOM	366	N	GLU	S	47	16.680	121.725	7.327	1.00	0.00
ATOM	367	CA	GLU	S	47	18.008	122.306	7.440	1.00	0.00
ATOM	368	C	GLU	S	47	19.157	121.726	6.653	1.00	0.00
ATOM	369	O	GLU	S	47	19.285	120.524	6.462	1.00	0.00
ATOM	370	CB	GLU	S	47	18.437	122.270	8.933	1.00	0.00
ATOM	371	CG	GLU	S	47	17.611	123.139	9.939	1.00	0.00
ATOM	372	CD	GLU	S	47	16.261	122.605	10.424	1.00	0.00
ATOM	373	OE1	GLU	S	47	15.865	121.475	10.175	1.00	0.00
ATOM	374	OE2	GLU	S	47	15.547	123.506	11.156	1.00	0.00
ATOM	375	N	PHE	S	48	20.009	122.619	6.241	1.00	0.00
ATOM	376	CA	PHE	S	48	21.214	122.238	5.567	1.00	0.00
ATOM	377	C	PHE	S	48	22.243	122.515	6.644	1.00	0.00
ATOM	378	O	PHE	S	48	22.276	123.633	7.151	1.00	0.00
ATOM	379	CB	PHE	S	48	21.486	123.120	4.329	1.00	0.00
ATOM	380	CG	PHE	S	48	20.461	122.941	3.240	1.00	0.00
ATOM	381	CD1	PHE	S	48	19.451	123.873	3.049	1.00	0.00
ATOM	382	CD2	PHE	S	48	20.396	121.768	2.510	1.00	0.00
ATOM	383	CE1	PHE	S	48	18.412	123.649	2.168	1.00	0.00
ATOM	384	CE2	PHE	S	48	19.356	121.533	1.629	1.00	0.00
ATOM	385	CZ	PHE	S	48	18.360	122.471	1.457	1.00	0.00
ATOM	386	N	LEU	S	49	23.057	121.530	6.983	1.00	0.00
ATOM	387	CA	LEU	S	49	24.204	121.685	7.871	1.00	0.00
ATOM	388	C	LEU	S	49	25.370	121.144	7.051	1.00	0.00
ATOM	389	O	LEU	S	49	25.182	120.088	6.465	1.00	0.00
ATOM	390	CB	LEU	S	49	24.120	120.835	9.146	1.00	0.00
ATOM	391	CG	LEU	S	49	23.058	120.927	10.250	1.00	0.00
ATOM	392	CD1	LEU	S	49	23.214	119.760	11.179	1.00	0.00
ATOM	393	CD2	LEU	S	49	23.186	122.224	11.006	1.00	0.00
ATOM	394	N	GLU	S	50	26.526	121.769	6.935	1.00	0.00
ATOM	395	CA	GLU	S	50	27.653	121.249	6.174	1.00	0.00
ATOM	396	C	GLU	S	50	28.112	119.861	6.627	1.00	0.00
ATOM	397	O	GLU	S	50	28.510	119.040	5.816	1.00	0.00
ATOM	398	CB	GLU	S	50	28.830	122.257	6.285	1.00	0.00
ATOM	399	CG	GLU	S	50	29.569	122.348	7.662	1.00	0.00
ATOM	400	CD	GLU	S	50	30.762	123.300	7.784	1.00	0.00
ATOM	401	OE1	GLU	S	50	30.776	124.418	7.286	1.00	0.00
ATOM	402	OE2	GLU	S	50	31.803	122.785	8.497	1.00	0.00
ATOM	403	N	PRO	S	51	28.122	119.585	7.912	1.00	0.00
ATOM	404	CA	PRO	S	51	28.568	118.309	8.440	1.00	0.00
ATOM	405	C	PRO	S	51	27.907	118.154	9.800	1.00	0.00
ATOM	406	O	PRO	S	51	27.068	118.983	10.147	1.00	0.00

ATOM	407	CB	PRO	S	51	30.082	118.404	8.562	1.00	0.00
ATOM	408	CG	PRO	S	51	30.249	119.892	8.916	1.00	0.00
ATOM	409	CD	PRO	S	51	29.235	120.596	8.008	1.00	0.00
ATOM	410	N	ALA	S	52	28.317	117.163	10.597	1.00	0.00
ATOM	411	CA	ALA	S	52	27.769	116.957	11.928	1.00	0.00
ATOM	412	C	ALA	S	52	28.283	117.934	12.969	1.00	0.00
ATOM	413	O	ALA	S	52	27.808	117.928	14.093	1.00	0.00
ATOM	414	CB	ALA	S	52	28.067	115.503	12.334	1.00	0.00
ATOM	415	N	ALA	S	53	29.238	118.789	12.642	1.00	0.00
ATOM	416	CA	ALA	S	53	29.828	119.729	13.593	1.00	0.00
ATOM	417	C	ALA	S	53	28.966	120.901	14.020	1.00	0.00
ATOM	418	O	ALA	S	53	29.248	121.555	15.025	1.00	0.00
ATOM	419	CB	ALA	S	53	31.100	120.269	12.997	1.00	0.00
ATOM	420	N	ASN	S	54	27.938	121.125	13.233	1.00	0.00
ATOM	421	CA	ASN	S	54	27.139	122.311	13.353	1.00	0.00
ATOM	422	C	ASN	S	54	25.720	121.889	13.574	1.00	0.00
ATOM	423	O	ASN	S	54	25.320	120.796	13.198	1.00	0.00
ATOM	424	CB	ASN	S	54	27.304	123.182	12.073	1.00	0.00
ATOM	425	CG	ASN	S	54	28.645	123.902	11.893	1.00	0.00
ATOM	426	OD1	ASN	S	54	29.199	124.489	12.811	1.00	0.00
ATOM	427	ND2	ASN	S	54	29.202	123.908	10.712	1.00	0.00
ATOM	428	N	GLU	S	55	24.957	122.785	14.131	1.00	0.00
ATOM	429	CA	GLU	S	55	23.562	122.559	14.366	1.00	0.00
ATOM	430	C	GLU	S	55	23.017	123.972	14.322	1.00	0.00
ATOM	431	O	GLU	S	55	23.695	124.903	14.755	1.00	0.00
ATOM	432	CB	GLU	S	55	23.446	121.921	15.712	1.00	0.00
ATOM	433	CG	GLU	S	55	22.134	121.248	15.915	1.00	0.00
ATOM	434	CD	GLU	S	55	21.996	120.761	17.336	1.00	0.00
ATOM	435	OE1	GLU	S	55	22.809	119.900	17.738	1.00	0.00
ATOM	436	OE2	GLU	S	55	21.076	121.241	18.045	1.00	0.00
ATOM	437	N	THR	S	56	21.838	124.153	13.769	1.00	0.00
ATOM	438	CA	THR	S	56	21.264	125.474	13.654	1.00	0.00
ATOM	439	C	THR	S	56	20.121	125.411	14.668	1.00	0.00
ATOM	440	O	THR	S	56	19.353	124.458	14.714	1.00	0.00
ATOM	441	CB	THR	S	56	20.837	125.761	12.175	1.00	0.00
ATOM	442	OG1	THR	S	56	19.788	124.886	11.780	1.00	0.00
ATOM	443	CG2	THR	S	56	21.927	125.569	11.099	1.00	0.00
ATOM	444	N	VAL	S	57	20.104	126.347	15.577	1.00	0.00
ATOM	445	CA	VAL	S	57	19.133	126.367	16.643	1.00	0.00
ATOM	446	C	VAL	S	57	17.815	127.012	16.227	1.00	0.00
ATOM	447	O	VAL	S	57	17.732	127.733	15.228	1.00	0.00
ATOM	448	CB	VAL	S	57	19.729	127.122	17.897	1.00	0.00
ATOM	449	CG1	VAL	S	57	20.993	126.492	18.545	1.00	0.00
ATOM	450	CG2	VAL	S	57	20.106	128.598	17.619	1.00	0.00
ATOM	451	N	ARG	S	58	16.811	126.805	17.069	1.00	0.00
ATOM	452	CA	ARG	S	58	15.471	127.333	16.873	1.00	0.00
ATOM	453	C	ARG	S	58	15.379	128.801	16.474	1.00	0.00
ATOM	454	O	ARG	S	58	14.522	129.186	15.698	1.00	0.00
ATOM	455	CB	ARG	S	58	14.650	127.120	18.174	1.00	0.00
ATOM	456	CG	ARG	S	58	14.494	125.641	18.615	1.00	0.00
ATOM	457	CD	ARG	S	58	13.787	124.784	17.559	1.00	0.00
ATOM	458	NE	ARG	S	58	13.880	123.361	17.972	1.00	0.00
ATOM	459	CZ	ARG	S	58	13.382	122.330	17.304	1.00	0.00
ATOM	460	NH1	ARG	S	58	12.751	122.426	16.171	1.00	0.00
ATOM	461	NH2	ARG	S	58	13.536	121.159	17.815	1.00	0.00
ATOM	462	N	HIS	S	59	16.260	129.637	16.972	1.00	0.00
ATOM	463	CA	HIS	S	59	16.193	131.077	16.775	1.00	0.00
ATOM	464	C	HIS	S	59	17.168	131.530	15.718	1.00	0.00
ATOM	465	O	HIS	S	59	17.465	132.722	15.606	1.00	0.00
ATOM	466	CB	HIS	S	59	16.542	131.716	18.129	1.00	0.00
ATOM	467	CG	HIS	S	59	15.498	131.447	19.173	1.00	0.00

ATOM	468	ND1	HIS	S	59	15.546	130.438	20.132	1.00	0.00
ATOM	469	CD2	HIS	S	59	14.338	132.201	19.287	1.00	0.00
ATOM	470	CE1	HIS	S	59	14.384	130.673	20.771	1.00	0.00
ATOM	471	NE2	HIS	S	59	13.606	131.698	20.330	1.00	0.00
ATOM	472	N	GLY	S	60	17.710	130.556	15.011	1.00	0.00
ATOM	473	CA	GLY	S	60	18.690	130.814	13.987	1.00	0.00
ATOM	474	C	GLY	S	60	20.126	130.874	14.458	1.00	0.00
ATOM	475	O	GLY	S	60	20.991	131.171	13.630	1.00	0.00
ATOM	476	N	CYS	S	61	20.463	130.621	15.715	1.00	0.00
ATOM	477	CA	CYS	S	61	21.841	130.709	16.186	1.00	0.00
ATOM	478	C	CYS	S	61	22.709	129.572	15.685	1.00	0.00
ATOM	479	O	CYS	S	61	22.215	128.442	15.408	1.00	0.00
ATOM	480	CB	CYS	S	61	21.818	130.701	17.718	1.00	0.00
ATOM	481	SG	CYS	S	61	20.269	131.378	18.384	1.00	0.00
ATOM	482	N	LEU	S	62	24.013	129.867	15.530	1.00	0.00
ATOM	483	CA	LEU	S	62	24.923	128.870	14.977	1.00	0.00
ATOM	484	C	LEU	S	62	25.707	128.127	16.036	1.00	0.00
ATOM	485	O	LEU	S	62	26.381	128.755	16.891	1.00	0.00
ATOM	486	CB	LEU	S	62	25.893	129.589	14.039	1.00	0.00
ATOM	487	CG	LEU	S	62	26.804	128.630	13.288	1.00	0.00
ATOM	488	CD1	LEU	S	62	26.039	127.742	12.305	1.00	0.00
ATOM	489	CD2	LEU	S	62	27.866	129.366	12.471	1.00	0.00
ATOM	490	N	ARG	S	63	25.721	126.828	16.123	1.00	0.00
ATOM	491	CA	ARG	S	63	26.508	126.175	17.209	1.00	0.00
ATOM	492	C	ARG	S	63	27.689	125.418	16.610	1.00	0.00
ATOM	493	O	ARG	S	63	27.584	124.833	15.556	1.00	0.00
ATOM	494	CB	ARG	S	63	25.569	125.241	18.020	1.00	0.00
ATOM	495	CG	ARG	S	63	26.160	124.717	19.355	1.00	0.00
ATOM	496	CD	ARG	S	63	25.201	123.777	20.095	1.00	0.00
ATOM	497	NE	ARG	S	63	25.845	123.357	21.365	1.00	0.00
ATOM	498	CZ	ARG	S	63	25.311	122.546	22.268	1.00	0.00
ATOM	499	NH1	ARG	S	63	24.136	121.999	22.159	1.00	0.00
ATOM	500	NH2	ARG	S	63	26.005	122.289	23.320	1.00	0.00
ATOM	501	N	LEU	S	64	28.815	125.420	17.269	1.00	0.00
ATOM	502	CA	LEU	S	64	29.982	124.680	16.701	1.00	0.00
ATOM	503	C	LEU	S	64	30.433	123.573	17.651	1.00	0.00
ATOM	504	O	LEU	S	64	30.865	123.817	18.759	1.00	0.00
ATOM	505	CB	LEU	S	64	31.149	125.660	16.388	1.00	0.00
ATOM	506	CG	LEU	S	64	30.904	126.777	15.339	1.00	0.00
ATOM	507	CD1	LEU	S	64	32.068	127.778	15.352	1.00	0.00
ATOM	508	CD2	LEU	S	64	30.711	126.226	13.916	1.00	0.00
ATOM	509	N	ASN	S	65	30.335	122.352	17.208	1.00	0.00
ATOM	510	CA	ASN	S	65	30.756	121.203	18.054	1.00	0.00
ATOM	511	C	ASN	S	65	31.594	120.243	17.214	1.00	0.00
ATOM	512	O	ASN	S	65	31.485	120.215	16.005	1.00	0.00
ATOM	513	CB	ASN	S	65	29.497	120.502	18.643	1.00	0.00
ATOM	514	CG	ASN	S	65	28.698	121.281	19.694	1.00	0.00
ATOM	515	OD1	ASN	S	65	27.484	121.408	19.628	1.00	0.00
ATOM	516	ND2	ASN	S	65	29.339	121.847	20.681	1.00	0.00
ATOM	517	N	GLN	S	66	32.431	119.459	17.836	1.00	0.00
ATOM	518	CA	GLN	S	66	33.277	118.504	17.064	1.00	0.00
ATOM	519	C	GLN	S	66	33.958	119.220	15.894	1.00	0.00
ATOM	520	O	GLN	S	66	34.271	118.629	14.880	1.00	0.00
ATOM	521	CB	GLN	S	66	32.388	117.343	16.569	1.00	0.00
ATOM	522	CG	GLN	S	66	31.824	116.384	17.671	1.00	0.00
ATOM	523	CD	GLN	S	66	32.803	115.543	18.500	1.00	0.00
ATOM	524	OE1	GLN	S	66	33.757	114.990	17.976	1.00	0.00
ATOM	525	NE2	GLN	S	66	32.607	115.388	19.785	1.00	0.00
ATOM	526	N	PRO	S	67	34.186	120.493	16.036	1.00	0.00
ATOM	527	CA	PRO	S	67	34.845	121.264	14.942	1.00	0.00
ATOM	528	C	PRO	S	67	36.275	120.760	14.725	1.00	0.00

ATOM	529	O	PRO	S	67	37.129	120.891	15.579	1.00	0.00
ATOM	530	CB	PRO	S	67	34.873	122.715	15.401	1.00	0.00
ATOM	531	CG	PRO	S	67	35.050	122.534	16.918	1.00	0.00
ATOM	532	CD	PRO	S	67	34.127	121.358	17.252	1.00	0.00
ATOM	533	N	THR	S	68	36.537	120.186	13.583	1.00	0.00
ATOM	534	CA	THR	S	68	37.907	119.672	13.295	1.00	0.00
ATOM	535	C	THR	S	68	38.803	120.815	12.809	1.00	0.00
ATOM	536	O	THR	S	68	38.495	121.976	12.993	1.00	0.00
ATOM	537	CB	THR	S	68	37.849	118.510	12.247	1.00	0.00
ATOM	538	OG1	THR	S	68	37.384	118.991	10.993	1.00	0.00
ATOM	539	CG2	THR	S	68	36.909	117.332	12.583	1.00	0.00
ATOM	540	N	HIS	S	69	39.911	120.501	12.193	1.00	0.00
ATOM	541	CA	HIS	S	69	40.821	121.575	11.700	1.00	0.00
ATOM	542	C	HIS	S	69	40.398	122.034	10.300	1.00	0.00
ATOM	543	O	HIS	S	69	41.207	122.132	9.399	1.00	0.00
ATOM	544	CB	HIS	S	69	42.232	120.965	11.667	1.00	0.00
ATOM	545	CG	HIS	S	69	42.703	120.545	13.028	1.00	0.00
ATOM	546	ND1	HIS	S	69	43.302	121.374	13.975	1.00	0.00
ATOM	547	CD2	HIS	S	69	42.588	119.243	13.494	1.00	0.00
ATOM	548	CE1	HIS	S	69	43.500	120.484	14.966	1.00	0.00
ATOM	549	NE2	HIS	S	69	43.108	119.197	14.760	1.00	0.00
ATOM	550	N	VAL	S	70	39.139	122.318	10.111	1.00	0.00
ATOM	551	CA	VAL	S	70	38.668	122.773	8.772	1.00	0.00
ATOM	552	C	VAL	S	70	37.381	123.571	8.920	1.00	0.00
ATOM	553	O	VAL	S	70	36.420	123.376	8.202	1.00	0.00
ATOM	554	CB	VAL	S	70	38.468	121.526	7.822	1.00	0.00
ATOM	555	CG1	VAL	S	70	37.249	120.613	8.131	1.00	0.00
ATOM	556	CG2	VAL	S	70	38.333	121.894	6.325	1.00	0.00
ATOM	557	N	ASN	S	71	37.361	124.459	9.860	1.00	0.00
ATOM	558	CA	ASN	S	71	36.151	125.285	10.098	1.00	0.00
ATOM	559	C	ASN	S	71	36.556	126.725	10.427	1.00	0.00
ATOM	560	O	ASN	S	71	35.899	127.661	10.024	1.00	0.00
ATOM	561	CB	ASN	S	71	35.312	124.659	11.250	1.00	0.00
ATOM	562	CG	ASN	S	71	34.860	123.205	11.067	1.00	0.00
ATOM	563	OD1	ASN	S	71	35.437	122.271	11.605	1.00	0.00
ATOM	564	ND2	ASN	S	71	33.838	122.956	10.293	1.00	0.00
ATOM	565	N	ASN	S	72	37.630	126.888	11.162	1.00	0.00
ATOM	566	CA	ASN	S	72	38.123	128.248	11.557	1.00	0.00
ATOM	567	C	ASN	S	72	36.982	129.099	12.106	1.00	0.00
ATOM	568	O	ASN	S	72	35.836	128.696	12.127	1.00	0.00
ATOM	569	CB	ASN	S	72	38.786	128.938	10.329	1.00	0.00
ATOM	570	CG	ASN	S	72	40.146	128.392	9.878	1.00	0.00
ATOM	571	OD1	ASN	S	72	41.034	128.119	10.673	1.00	0.00
ATOM	572	ND2	ASN	S	72	40.368	128.233	8.601	1.00	0.00
ATOM	573	N	GLY	S	73	37.299	130.248	12.613	1.00	0.00
ATOM	574	CA	GLY	S	73	36.274	131.093	13.211	1.00	0.00
ATOM	575	C	GLY	S	73	35.613	132.016	12.232	1.00	0.00
ATOM	576	O	GLY	S	73	34.541	131.721	11.771	1.00	0.00
ATOM	577	N	ASN	S	74	36.203	133.166	12.011	1.00	0.00
ATOM	578	CA	ASN	S	74	35.600	134.226	11.123	1.00	0.00
ATOM	579	C	ASN	S	74	34.320	133.736	10.423	1.00	0.00
ATOM	580	O	ASN	S	74	34.345	133.328	9.285	1.00	0.00
ATOM	581	CB	ASN	S	74	36.675	134.574	10.090	1.00	0.00
ATOM	582	CG	ASN	S	74	37.814	135.372	10.736	1.00	0.00
ATOM	583	OD1	ASN	S	74	37.577	136.278	11.507	1.00	0.00
ATOM	584	ND2	ASN	S	74	39.051	135.085	10.430	1.00	0.00
ATOM	585	N	TYR	S	75	33.214	133.745	11.128	1.00	0.00
ATOM	586	CA	TYR	S	75	31.932	133.248	10.537	1.00	0.00
ATOM	587	C	TYR	S	75	30.924	134.381	10.416	1.00	0.00
ATOM	588	O	TYR	S	75	30.723	135.125	11.352	1.00	0.00
ATOM	589	CB	TYR	S	75	31.389	132.210	11.525	1.00	0.00

ATOM	590	CG	TYR	S	75	31.029	132.854	12.841	1.00	0.00
ATOM	591	CD1	TYR	S	75	31.928	132.852	13.914	1.00	0.00
ATOM	592	CD2	TYR	S	75	29.768	133.434	12.988	1.00	0.00
ATOM	593	CE1	TYR	S	75	31.555	133.433	15.133	1.00	0.00
ATOM	594	CE2	TYR	S	75	29.395	134.018	14.201	1.00	0.00
ATOM	595	CZ	TYR	S	75	30.288	134.017	15.277	1.00	0.00
ATOM	596	OH	TYR	S	75	29.922	134.591	16.479	1.00	0.00
ATOM	597	N	THR	S	76	30.267	134.523	9.294	1.00	0.00
ATOM	598	CA	THR	S	76	29.263	135.622	9.197	1.00	0.00
ATOM	599	C	THR	S	76	27.880	135.051	8.903	1.00	0.00
ATOM	600	O	THR	S	76	27.708	133.862	8.764	1.00	0.00
ATOM	601	CB	THR	S	76	29.688	136.660	8.104	1.00	0.00
ATOM	602	OG1	THR	S	76	29.678	136.060	6.816	1.00	0.00
ATOM	603	CG2	THR	S	76	31.106	137.257	8.241	1.00	0.00
ATOM	604	N	LEU	S	77	26.895	135.893	8.806	1.00	0.00
ATOM	605	CA	LEU	S	77	25.515	135.387	8.507	1.00	0.00
ATOM	606	C	LEU	S	77	24.785	136.340	7.564	1.00	0.00
ATOM	607	O	LEU	S	77	24.383	137.418	7.948	1.00	0.00
ATOM	608	CB	LEU	S	77	24.714	135.178	9.824	1.00	0.00
ATOM	609	CG	LEU	S	77	23.375	134.397	9.749	1.00	0.00
ATOM	610	CD1	LEU	S	77	22.522	134.691	10.990	1.00	0.00
ATOM	611	CD2	LEU	S	77	22.569	134.718	8.478	1.00	0.00
ATOM	612	N	LEU	S	78	24.574	135.940	6.346	1.00	0.00
ATOM	613	CA	LEU	S	78	23.836	136.828	5.407	1.00	0.00
ATOM	614	C	LEU	S	78	22.340	136.546	5.563	1.00	0.00
ATOM	615	O	LEU	S	78	21.873	135.471	5.253	1.00	0.00
ATOM	616	CB	LEU	S	78	24.334	136.614	3.949	1.00	0.00
ATOM	617	CG	LEU	S	78	23.747	137.522	2.835	1.00	0.00
ATOM	618	CD1	LEU	S	78	24.182	138.977	3.057	1.00	0.00
ATOM	619	CD2	LEU	S	78	24.153	137.068	1.423	1.00	0.00
ATOM	620	N	ALA	S	79	21.586	137.484	6.062	1.00	0.00
ATOM	621	CA	ALA	S	79	20.136	137.228	6.264	1.00	0.00
ATOM	622	C	ALA	S	79	19.299	138.114	5.342	1.00	0.00
ATOM	623	O	ALA	S	79	19.414	139.320	5.360	1.00	0.00
ATOM	624	CB	ALA	S	79	19.827	137.450	7.755	1.00	0.00
ATOM	625	N	ALA	S	80	18.462	137.525	4.534	1.00	0.00
ATOM	626	CA	ALA	S	80	17.632	138.344	3.606	1.00	0.00
ATOM	627	C	ALA	S	80	16.162	138.328	4.028	1.00	0.00
ATOM	628	O	ALA	S	80	15.835	138.089	5.173	1.00	0.00
ATOM	629	CB	ALA	S	80	17.851	137.801	2.183	1.00	0.00
ATOM	630	N	ASN	S	81	15.273	138.588	3.109	1.00	0.00
ATOM	631	CA	ASN	S	81	13.826	138.599	3.447	1.00	0.00
ATOM	632	C	ASN	S	81	12.992	138.709	2.165	1.00	0.00
ATOM	633	O	ASN	S	81	13.512	138.953	1.094	1.00	0.00
ATOM	634	CB	ASN	S	81	13.647	139.840	4.330	1.00	0.00
ATOM	635	CG	ASN	S	81	12.241	139.858	4.940	1.00	0.00
ATOM	636	OD1	ASN	S	81	11.527	138.878	4.881	1.00	0.00
ATOM	637	ND2	ASN	S	81	11.813	140.943	5.527	1.00	0.00
ATOM	638	N	PRO	S	82	11.700	138.537	2.267	1.00	0.00
ATOM	639	CA	PRO	S	82	10.829	138.635	1.056	1.00	0.00
ATOM	640	C	PRO	S	82	11.132	139.908	0.270	1.00	0.00
ATOM	641	O	PRO	S	82	10.885	139.996	-0.915	1.00	0.00
ATOM	642	CB	PRO	S	82	9.395	138.679	1.565	1.00	0.00
ATOM	643	CG	PRO	S	82	9.582	139.447	2.885	1.00	0.00
ATOM	644	CD	PRO	S	82	10.872	138.858	3.466	1.00	0.00
ATOM	645	N	PHE	S	83	11.666	140.888	0.925	1.00	0.00
ATOM	646	CA	PHE	S	83	11.997	142.161	0.236	1.00	0.00
ATOM	647	C	PHE	S	83	13.088	142.907	1.009	1.00	0.00
ATOM	648	O	PHE	S	83	12.984	144.091	1.260	1.00	0.00
ATOM	649	CB	PHE	S	83	10.727	143.061	0.136	1.00	0.00
ATOM	650	CG	PHE	S	83	9.587	142.538	-0.750	1.00	0.00

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ATOM    651  CD1 PHE S  83       8.465 141.947  -0.158  1.00  0.00
ATOM    652  CD2 PHE S  83       9.683 142.588  -2.144  1.00  0.00
ATOM    653  CE1 PHE S  83       7.462 141.397  -0.948  1.00  0.00
ATOM    654  CE2 PHE S  83       8.672 142.047  -2.934  1.00  0.00
ATOM    655  CZ  PHE S  83       7.564 141.449  -2.337  1.00  0.00
ATOM    656  N   GLY S  84      14.133 142.222   1.390  1.00  0.00
ATOM    657  CA  GLY S  84      15.226 142.895   2.147  1.00  0.00
ATOM    658  C   GLY S  84      16.530 142.112   1.980  1.00  0.00
ATOM    659  O   GLY S  84      16.578 141.094   1.320  1.00  0.00
ATOM    660  N   GLN S  85      17.591 142.583   2.579  1.00  0.00
ATOM    661  CA  GLN S  85      18.899 141.876   2.463  1.00  0.00
ATOM    662  C   GLN S  85      19.900 142.466   3.460  1.00  0.00
ATOM    663  O   GLN S  85      20.044 143.668   3.562  1.00  0.00
ATOM    664  CB  GLN S  85      19.404 142.004   1.009  1.00  0.00
ATOM    665  CG  GLN S  85      20.641 141.124   0.627  1.00  0.00
ATOM    666  CD  GLN S  85      20.483 139.601   0.566  1.00  0.00
ATOM    667  OE1 GLN S  85      21.199 138.866   1.227  1.00  0.00
ATOM    668  NE2 GLN S  85      19.587 139.067  -0.225  1.00  0.00
ATOM    669  N   ALA S  86      20.592 141.639   4.200  1.00  0.00
ATOM    670  CA  ALA S  86      21.573 142.168   5.183  1.00  0.00
ATOM    671  C   ALA S  86      22.594 141.092   5.513  1.00  0.00
ATOM    672  O   ALA S  86      22.628 140.043   4.898  1.00  0.00
ATOM    673  CB  ALA S  86      20.784 142.663   6.407  1.00  0.00
ATOM    674  N   SER S  87      23.422 141.334   6.481  1.00  0.00
ATOM    675  CA  SER S  87      24.434 140.314   6.849  1.00  0.00
ATOM    676  C   SER S  87      25.161 140.709   8.132  1.00  0.00
ATOM    677  O   SER S  87      25.118 141.842   8.570  1.00  0.00
ATOM    678  CB  SER S  87      25.423 140.073   5.681  1.00  0.00
ATOM    679  OG  SER S  87      26.219 141.224   5.379  1.00  0.00
ATOM    680  N   ALA S  88      25.833 139.771   8.723  1.00  0.00
ATOM    681  CA  ALA S  88      26.589 140.050   9.977  1.00  0.00
ATOM    682  C   ALA S  88      27.936 139.346   9.899  1.00  0.00
ATOM    683  O   ALA S  88      28.253 138.687   8.930  1.00  0.00
ATOM    684  CB  ALA S  88      25.790 139.514  11.147  1.00  0.00
ATOM    685  OXT ALA S  88      28.690 139.488  10.891  1.00  0.00
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